EXPERIMENTAL TAXONOMY IN THE GENUS <u>CAREX</u> SECTION <u>VESICARIAE</u>

by

George John Shepherd

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I declare that this thesis has been composed entirely by myself and the experiments and results described herein are a product of my own work.

SUMMARY

This thesis describes an experimental taxonomic study of the British species of *Carex* Sect. *Vesicariae*. A variety of techniques (anatomy, chemotaxonomy, biosystematics, cytology and morphology) were applied to three main problems:

1) the delimitation of Sect. *Vesicariae*
2) the sources of the widespread variation noted in many of the species of Sect. *Vesicariae*
3) the nature and origins of *C. grahamii*.

The delimitation of Sect. *Vesicariae* has been a source of controversy as some authors have claimed that the section should be extended to include species from related sections such as the *Pseudocypereae*, *Hirtae* and *Paludosae*. This proposal is supported by data from natural hybrids. Anatomical, biosystematic and cytological surveys in the present study suggest that there is little justification for extending the limits of Sect. *Vesicariae*. A consideration of published data on chromosome numbers supports this conclusion. The geographical distributions of the sections involved are also distinctive. A preliminary chemotaxonomic investigation using seed proteins has also been carried out. Attempts to produce antisera from *Carex* seed proteins were not very successful, but results from acrylamide gel electrophoresis were much more promising, showing a wide variety of banding patterns in different sections of *Carex*. Being of a preliminary nature, this survey was not, however, sufficiently complete to yield much information on sectional limits in Sect. *Vesicariae*.

Morphological and anatomical studies were made on *C. rostrata* to investigate the variation patterns occurring in this species. It would appear that a considerable part of the small-scale local variation is

genetical in origin. The evidence obtained suggests that C.rostrata forms extensive clones by vegetative spread. These may be morphologically very diverse and can sometimes be distinguished visually in the field. Studies on a population sample from Grantown-on- Spey, (Moray), indicate that hybridisation between C.rostrata and C.vesicaria can occur under natural conditions, and that some degree of backcrossing and introgression may be possible. Introgression is not, however, considered to be a significant source of variation in either species under normal conditions.

Studies of N.American material using anatomical and morphological techniques suggest that the taxon known as C.rostrata var. utriculata should probably be raised to subspecific or possibly specific rank. It is anatomically distinct from the type variety and a Principal Components Analysis (PCA) indicated that the two forms could be separated almost completely on morphological characters. The reported chromosome numbers for the two varieties also support the change of rank. No formal nomenclatural proposal has been made as further studies are required.

Material of C.grahamii from Britain and Scandinavia has been examined morphologically using PCA and a cytological and biosystematic study carried out on a clone from Meall nan Tarmachan in Perthshire. The chromosome number obtained was 2n = 82 or 84. This appears to be the first count for this taxon. Biosystematic and morphological evidence suggests that C.grahamii is a hybrid between C.saxatilis and C.vesicaria as suggested by a number of authors, but it is necessary to postulate some chromosomal rearrangements to account for the observed karyotype.

C.grahamii is rather heterogeneous and it is suggested that the taxon consists of a series of F_1 hybrids and backcrosses with C.saxatilis. The regular chromosome pairing seen in C.grahamii and in a C.grahamii x vesicaria hybrid would seem to indicate that C.saxatilis and C.vesicaria are very closely related. This is apparently confirmed by the similarity in the seed protein complements of the two species.

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CONTENTS

Between Pages

LIST OF FIGURES

Between Pages

CHAPTER 1 INTRODUCTION

I first became interested in the taxonomy of the genus Carex while attempting to identify Carex species in the field. The genus presents a number of difficulties for a beginner as the species are numerous (it is the largest non-apomictic genus in the British flora) and they are often separated by small technical differences which demand careful observation if correct identifications are to be made. The major problems are caused by the very reduced floral and inflorescence structures found in the Cyperaceae. Many of the floral organs which are normally used to distinguish species and genera are either entirely absent or reduced to minute structures which are difficult to observe and interpret. As a result, Carex has frequently been regarded as a 'difficult' genus which should be left to specialists. This is rather unfortunate since the majority of the species recognised are quite well defined, and there are few groups within the genus which can be regarded as 'critical'.

The taxonomic difficulties surrounding Carex are not, however, confined to the inexperienced who are studying the genus for the first time. Although there are comparatively few groups which raise problems at specific rank, the subgeneric and sectional classification of Carex is a subject which has given rise to much controversy. In the absence of many of the standard floral characters, delimitation of taxa at subgeneric and sectional level has depended largely on details of utricle and inflorescence structure. It is therefore very possible that much of this somewhat artificial infra-generic classification is unnatural, though there is little evidence available at present to indicate the extent to which this is so. These problems are increased by the large size of the genus. Estimates vary from ca.800 (Kükenthal, 1909) to 1,800 or more (Kreczetowicz, 1935). The latter estimate would make Carex comparable to Astragalus and Senecio in species numbers (cf. Good, 1964).

Kükenthal's work is the most recent monograph available for the whole genus. He recognised four subgenera and 69 sections within Carex, and these were further divided into subsections where necessary. About 800 species were described but it should be borne in mind that Kükenthal's species concept was quite broad and many taxa given specific rank by other authors were treated as subspecies or varieties. Kükenthal's subgenera, and in particular, subgenus Primocarex, present a good example of the difficulties surrounding any attempt to subdivide the genus. Kükenthal placed all the species with single undivided spikes in subgenus Primocarex and considered it to be the most primitive of his four subgenera, but this view has subsequently been challenged by Kreczetowicz (1936), Nelmes (1951), Kern & Reichgelt (1954), and Koyama (1962) who consider that subgenus Primocarex consists of a diverse assemblage of elements derived both from other subgenera of Carex and other distinct genera such as Uncinia and Kobresia (cf. Hamlin (1959) for a discussion of the relationships of these genera). It seems likely that Carex is in fact an unnatural group which contains species of widely differing origins and would probably be split into several genera were it not for the difficulty in distinguishing these groups easily and conveniently. At present, if species such as C.microglochin were to be placed in Uncinia, as Nelmes (1951) has suggested, there would be very few characters which could be used to separate the two genera, whereas retaining this species in Carex makes it possible to use the hooked rachilla of Uncinia as a generic character, however artificial this may be. There is therefore a great need for surveys of the entire subfamily

Caricoideae using a wider range of taxonomic techniques which might give enough additional information to allow some decision to be made on generic limits and relationships in this subfamily and *Carex* in particular. Taxonomic problems in the remaining subfamilies are mainly at generic level because of the greater diversity in structure in these groups, but additional data from experimental and anatomical investigations are badly needed to provide a more substantial basis for delimitation of the genera. Oteng-Yeboah (1972, 1974) discusses the difficulties faced by the taxonomist in dealing with the subfamily *Cyperoideae* and he has successfully applied anatomical studies to various problems within this subfamily, along with a reconsideration of much of the standard morphological evidence. The same considerations of course apply to the whole of the *Cyperaceae* since the very reduced nature of the flowers and inflorescences of this family and those which appear to be more or less closely related to it (e.g. *Gramineae*, *Juncaceae*) has made it almost impossible to decide with any certainty the origins and inter-relationships of these groups. Cronquist (1968) and Takhtajan (1969) give a summary of the recent position on this problem but it is obvious that there is as yet no general agreement.

Because of the difficulties besetting the use of conventional herbarium/morphological techniques, the *Cyperaceae* appear to offer an excellent subject for experimental taxonomic methods which may provide a more extensive data base for taxonomic decision making. The use of experimental approaches such as chemosystematics and biosystematics, in addition to cytology, numerical taxonomy and anatomical studies has in recent years vastly extended the amount of taxonomic data available for a wide range of plant groups. One of the objectives of the present study was to test a variety of experimental techniques on a small group of *Carex* species, bearing in mind the possibility that these methods might be applicable to a wider range of problems within the *Cyperaceae* as a whole.

The group chosen for study was section *Vesicariae* in subgenus *Carex*. The reasons for this choice were that:-

a) There are a number of taxonomic problems in this section, both at species and sectional levels, and it can therefore be investigated at several levels using a wide variety of techniques.

b) Several representatives of the section are native to the British Isles and N.W. Europe, making it possible to obtain live material for experimental studies without too much difficulty.

The systematic position of the group and a more detailed consideration of the problems being investigated are given in chapter 2, but the latter can be briefly summarised as follows:-

1) What are the limits of Sect. *Vesicariae*? Evidence from natural hybrids suggests that members of the *Vesicariae* are capable of forming hybrids with species from a number of other sections. Some authors consider that the members of the hybridising sections should all be included in a single large section.

2) What is the source of the widespread variation which has been noted in some of the species in Sect. *Vesicariae*? Can it be explained by hybridisation and introgression?

3) What are the relationships between the British species of the section, and in particular, what is the status of the taxon normally known as *C.grahamii*? The latter has been variously treated as a hybrid or a distinct species by different authors.

Although a detailed investigation using only one technique would have enabled production of a more complete survey of the wh ole section, it was decided to use a variety of methods to give as wide a range of information as possible. It was also hoped that a survey of this type would produce complementary sources of information, giving a less restricted viewpoint and providing more solid support for any conclusions which might be reached.

Comparatively few intensive studies of small groups of Carex species, using a variety of techniques, appear to have been carried out. Faulkner (1970, 1972, 1973) made a biosytematic study of the British material of Section Acutae, with an extensive survey of the cytology and crossability of the species in this section. He gives references for a large number of cytological studies in the Cyperaceae and discusses the problems of chromosome structure and movement in some detail. Davies (1955) also used cytological data in a study of sect. Flavae.

A large number of anatomical investigations have been carried out in Carex, and an extensive literature list is given in Metcalfe (1971). Although each species dealt with in Metcalfe's study is described in some detail, including the structure of roots, rhizome, leaves and culm, the total coverage (28 spp. out of a possible 1800) is insufficient to be able to draw many meaningful conclusions about the agreement, or otherwise, between anatomical and morphological data.

Biochemical studies have been limited mainly to broad surveys of flavonoid components which were not primarily concerned with Carex (e.g. Harborne, 1971), although Kukkonen (1971) has carried out a more detailed survey within the Cyperaceae. Virtually no other chemosystematic studies have been made.

The techniques employed can be grouped in four main categories as follows:-

1) Standard morphological techniques were used to investigate and describe the morphological variation in herbarium material and population samples. These studies form the basis for much of the work carried out at species and sub-specific levels, and by use of scatter diagrams (cf. Anderson, 1949) and other techniques, they can provide evidence for hybridisation and introgression. A discussion of the theoretical basis for these methods and examples of their use are given by Davis & Heywood (1963) and Radford *et al*. (1974). In addition, Principal Components Analysis (PCA) was applied to some of the morphological data. This multivariate statistical technique has been found to be extremely useful in reducing the number of variables required to describe a complex variation pattern and has been used extensively in a number of investigations (cf. Blackith & Reyment, 1971).

2) Anatomical studies were used to provide additional characters at both species and sectional levels. Metcalfe (1969, 1971) and Oteng-Yeboah (1972) have found that anatomical data provided valuable information in studies of other parts of the *Cyperaceae*, and the usefulness of such data in other families is well known e.g. in the *Gramineae* as described by Brown (1957). It was hoped that anatomical characters would be particularly useful at sectional level.

3) Chemosystematic methods were applied to seek information at sectional level and also to provide indications of the relationships between the species in the *Vesicariae*. Serological and gel-electrophoretic studies of seed proteins were used because proteins, in theory at least, should provide less ambiguous evidence of phylogenetic

relationships than the secondary plant products such as flavonoids. Serological techniques have been used with considerable success in a number of plant groups. Smith (1968, 1969, 1972) found that serological studies of seed proteins gave a valuable guide to relationships within Bromus (Gramineae) and Tucker (1969) has used these techniques to investigate the relationships between genera in the Solanaceae. Numerous other examples are given in Leone (1964) and Fairbrothers (1968). Gel electrophoresis techniques are more recent and have not been used quite so widely but the number of studies using these methods has increased enormously. Examples of taxonomic applications are given by Hunziker (1969).

4) Biosystematic and cytological methods provide a means of investigating relationships between taxa at specific and sectional levels and were therefore intended to clarify both the relationships between the species in the Vesicariae and the limits of the section. Biosystematic methods have now become standard when dealing with evolutionary and phenetic relationships between small species groups and in studies of intrageneric relationships. A good example is provided by Gajewski's work on Geum (Gajewski, 1959). Solbrig (1970) and Grant (1971) give numerous examples of biosystematic studies. Examples of the value of chromosome numbers and morphology are given by Stebbins (1971) and Moore (1968).

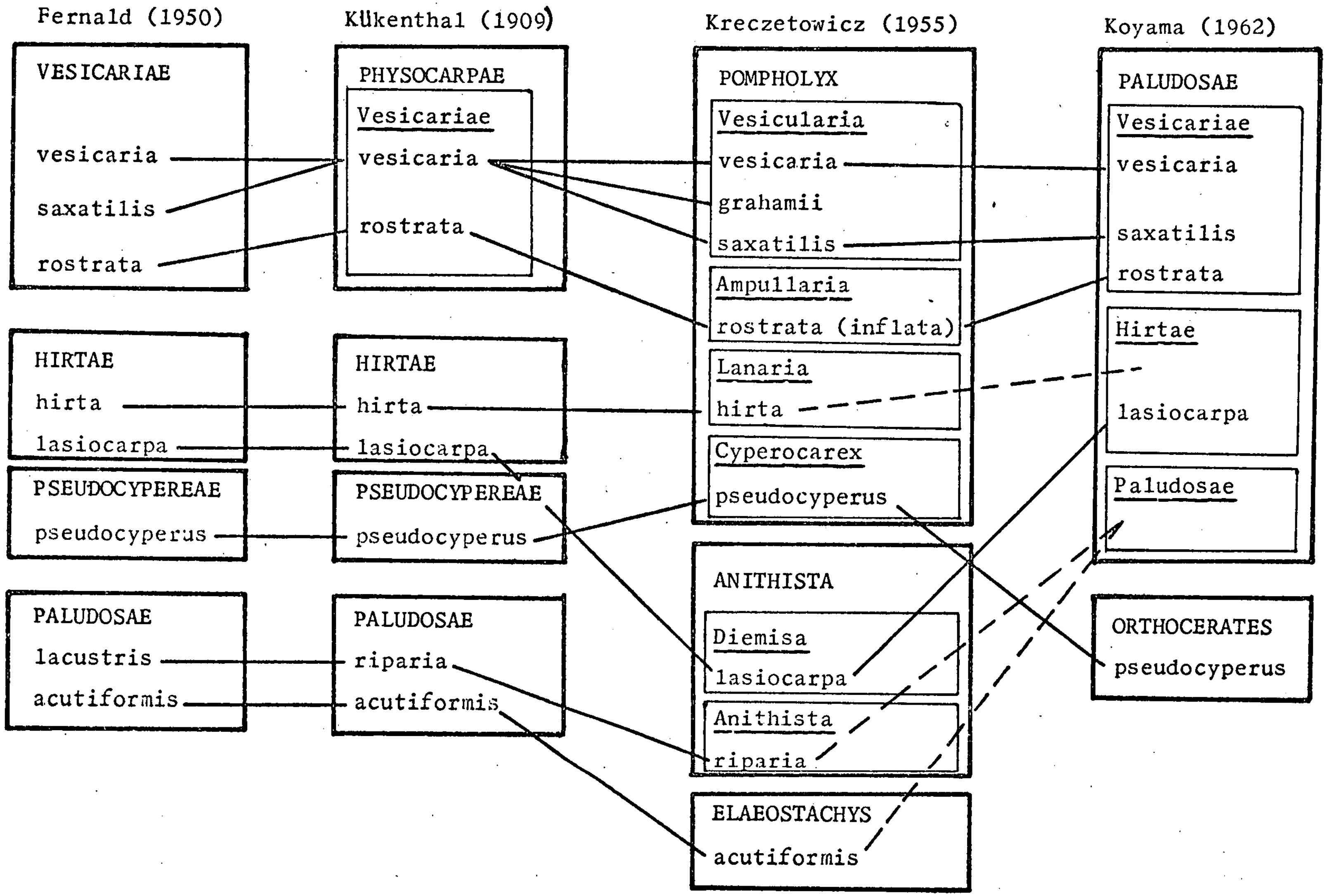
CHAPTER 2 THE SYSTEMATIC POSITION AND RELATIONSHIPS OF SECT. VESICARIAE

2.1 Taxonomic position of Sect.Vesicariae

Section Vesicariae, as used by Clapham, Tutin & Warburg (1962) forms a fairly well defined natural group as far as the four British species are concerned. Kükenthal (op.cit.) included it as a subsection of his Sect.Physocarpae, distinguishing it from the other subsections by the size, shape and surface texture of the utricles, but most N.American authors now treat this group as two separate sections, merging subsections Tentaculatae and Vesicariae to form Sect.Vesicariae and maintaining the Lupulinae as a section (Fernald, 1950; MacKenzie, 1931; Hermann, 1970). The species involved are mainly boreal or circumboreal, with a centre of diversity in N.America, though some species of Subsect.Tentaculatae reach South America (C.lurida Wahl., C.stenolepis Torr.) and the Himalayas (C.rostrata Stokes, C.obscuriceps Kük.) according to Kükenthal.

Kreczetowicz (1935), in his account of Carex for the Flora U.R.S.S. produced a rather different classification which he claimed was based partly on data from hybridisation and was therefore a more natural system. This is summarised in Fig.2.1 along with some of the other schemes which have been proposed. The main differences from the treatments by Kükenthal, MacKenzie and Fernald are that C.pseudocyperus L. and C.hirta L. are both included in the section (as Sect.Pompholyx Krecz.) and this is split into five 'cycles' (? subsections). Kükenthal's Hirtae and Paludosae are both split by inclusion of some of their species in different sections. Koyama (1962) in his revision of the Japanese Cyperaceae, combined sections

Fig.2.1 Summary of classification schemes proposed for the *Vesicariae* complex. (only British species are shown)



Fernald (1950)
Kükenthal (1909)
Kreczetowicz (1955)
Koyama (1962)
VESICARIAE
vesicaria
saxatilis
rostrata
HIRTAE
hirta
lasiocarpa
PSEUDOCYPEREAE
pseudocyperus
PALUDOSAE
lacustris
acutiformis
PHYSOCARPAE
Vesicariae
vesicaria
rostrata
HIRTAE
hirta
lasiocarpa
PSEUDOCYPEREAE
pseudocyperus
PALUDOSAE
riparia
acutiformis
POMPHOLYX
Vesicularia
vesicaria
grahamii
saxatilis
Ampullaria
rostrata (inflata)
Lanaria
hirta
Cyperocarex
pseudocyperus
ANITHISTA
Diemisa
lasiocarpa
Anithista
riparia
ELAEOSTACHYS
acutiformis
PALUDOSAE
Vesicariae
vesicaria
saxatilis
rostrata
Hirtae
lasiocarpa
Paludosae
ORTHOCERATES
pseudocyperus

Lupulinae, Vesicariae, Hirtae and Paludosae under Sect.Paludosae, retaining them as series within the section, while C.pseudocyperus is placed in sect. Orthocerates Koch. (which also contains C.pauciflora Lightf., one of Kükenthal's Primocarex species). Koyama's treatment is therefore more extreme than that of Kreczetowicz and unites three of the latter's sections.

A consideration of the patterns of natural hybridization in different sections of subgenus Carex would seem to lend some support to the views of Kreczetowicz and Koyama. Fig.2.2 shows the natural hybrids reported for the British species of this subgenus. From this it can be seen that a number of species complexes can be distinguished since their members tend to hybridise. Sections Paludosae, Hirtae, Pseudocypereae and Vesicariae form one complex, with apparently extensive hybridisation occurring between many of the species in these four sections. The Acutae seem to form a well marked separate group and, although a hybrid is reported between C.acutiformis and C.acuta, it seems reasonable to regard the Acutae as being quite distinct. Another complex is formed by the members of Sections Spirostachyae, Flavae and **Elatae**. The other British sections are not linked by hybridisation, with only intrasectional hybrids reported and none between sections. There would therefore seem to be some support for Kreczetowicz's scheme of including C.hirta and C.pseudocyperus in one section with the Vesicariae, but on this basis it would seem necessary to include the other members of sections Hirtae and Paludosae as is done by Koyama. There appears to be no obvious basis, on hybridisation grounds at least, for the splitting of Sect.Hirtae since C.lasiocarpa was thought by Kreczetowicz to hybridise with both C.rostrata and C.vesicaria as does C.hirta.

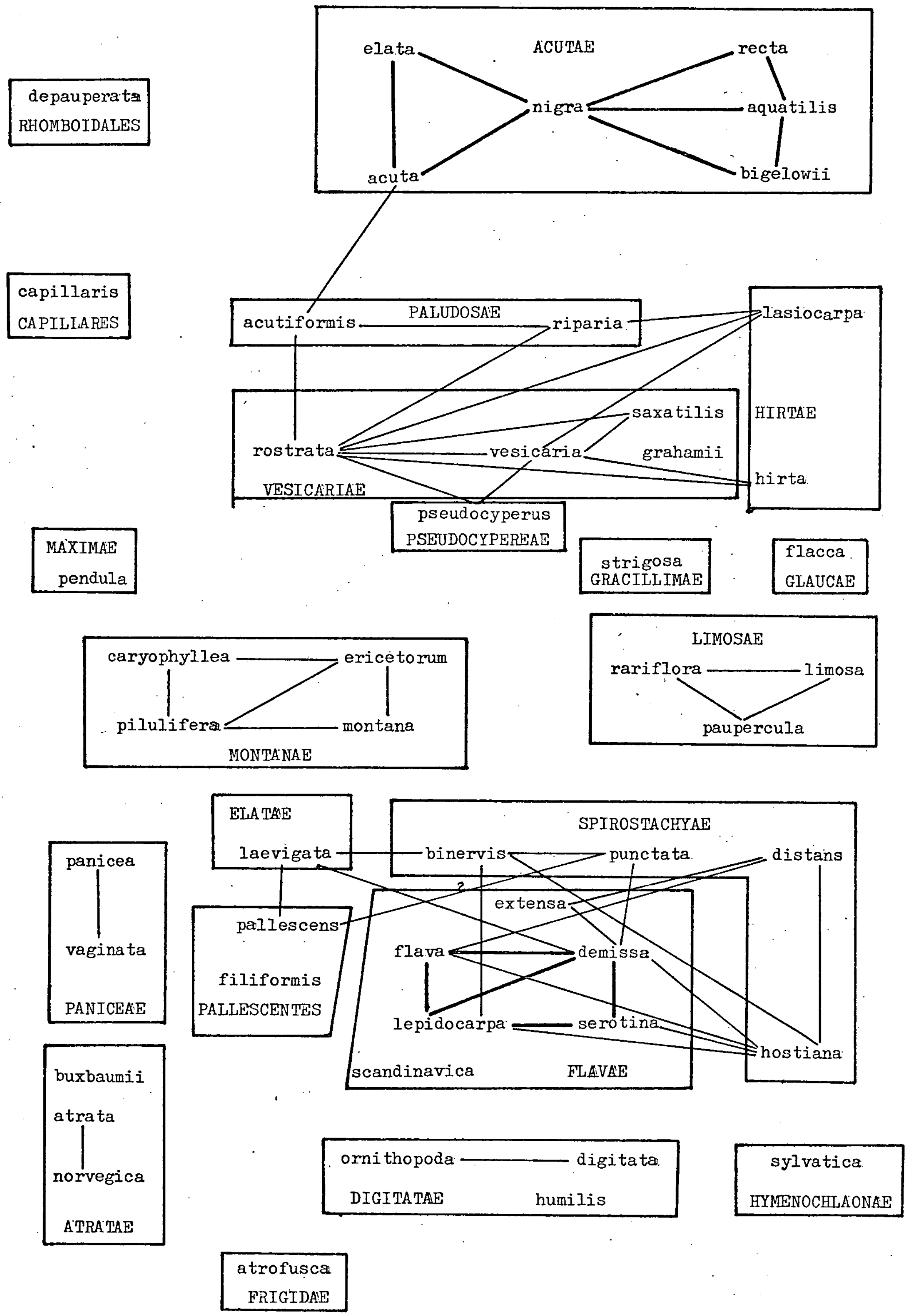
Fig. 2.2 Reported natural hybrids in British species of subgenus <u>Carex</u>.

Fig. 2.3 Reported natural hybrids in British species of subgenus <u>Vignea</u>.

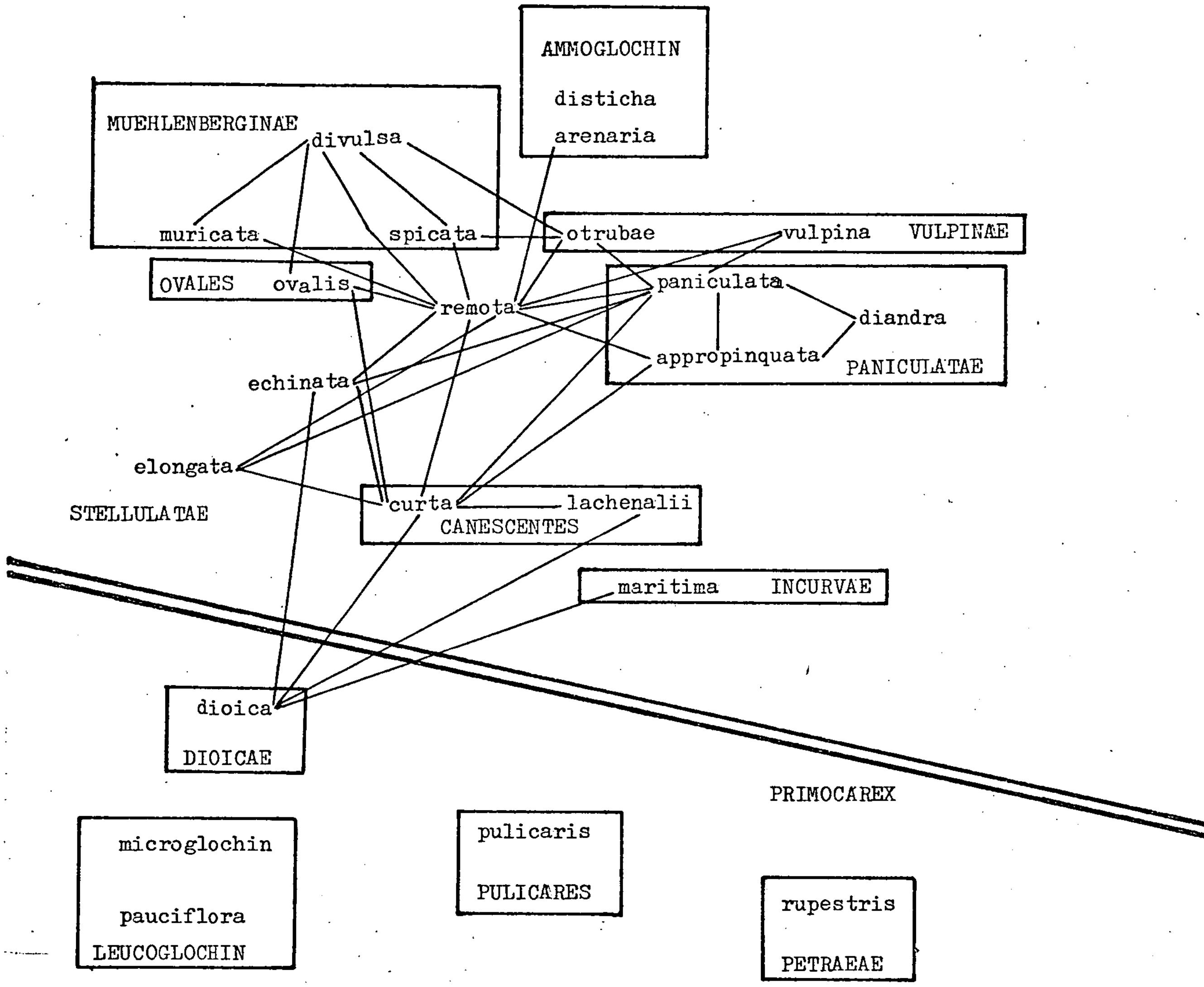
sterile hybrids

fertile hybrids

based on information from Jermy & Tutin (1968), Clapham, Tutin & Warburg (1962), David (1974) & [illegible]



ACUTAE
elata
recta
nigra
aquatilis
acuta
bigelowii
depauperata
RHOMBOIDALES
capillaris
CAPILLARES
PALUDOSAE
acutiformis
riparia
lasiocarpa
HIRTAE
saxatilis
rostrata
vesicaria
grahamii
hirta
VESICARIAE
pseudocyperus
PSEUDOCYPEREAE
MAXIMAE
pendula
strigosa
GRACILLIMAE
flacca
GLAUCAE
LIMOSAE
rariflora
limosa
paupercula
caryophyllea
ericetorum
pilulifera
montana
MONTANAE
ELATAE
laevigata
SPIROSTACHYAE
binervis
punctata
distans
panicea
vaginata
PANICEAE
?
extensa
pallescens
filiformis
PALLESCENTES
flava
demissa
lepidocarpa
serotina
hostiana
scandinavica
FLAVAE
buxbaumii
atrata
norvegica
ATRATAE
ornithopoda
digitata
DIGITATAE
humilis
sylvatica
HYMENOCHLAONAE
atrofusca
FRIGIDAE



AMMOGLOCHIN
disticha
arenaria
MUEHLENBERGINAE
divulsa
muricata
spicata
otrubae
vulpina
VULPINAE
OVALES
ovalis
remota
paniculata
diandra
appropinquata
PANICULATAE
echinata
elongata
STELLULATAE
curta
lachenalii
CANESCENTES
maritima
INCURVAE
dioica
DIOICAE
PRIMOCAREX
microglochin
pauciflora
LEUCOGLOCHIN
pulicaris
PULICARES
rupestris
PETRAEAE

It is also of interest that hybridisation between sections of subgenus Vignea (at least as far as the British species are concerned) is much more extensive than in subgenus Carex and, as a result, it is not possible to pick out species groups in the same way (Fig.2.3). The position of C.dioica is particularly interesting, and the cross-ability data certainly seem to support its transfer to subgenus Vignea as proposed by Kreczetowicz (op.cit.) and Kern and Reichgelt (1954). It must, however, be borne in mind that these data are not very reliable since -

a) only natural hybrids are reported here and the list is not necessarily complete - it is entirely possible that a number of other hybrids occur but have not beenfound or recognised. In addition, many of the species have widely separated habitats and geographical ranges so that cross-pollination must be an extremely rare event.

b) many so-called 'hybrids' are probably odd variants of one or other of the parents rather than true hybrids, so that the relationships suggested in Fig.2.3 may in some cases be non-existent.

In spite of these reservations, there would seem to be some evidence to indicate that the sections in the Vesicariae complex are rather more closely related to each other than to any of the other sections in the genus and it might therefore be more appropriate to consider these as members of a single section if this category is to be used in a consistent way throughout the genus.

It is of interest that Saville & Calder (1953) have proposed that all the sections of the Vesicariae complex should be included in a new subgenus which they named Kuekenthalia in honour of Kükenthal. This conclusion was reached on the basis of data from the distribution of smut fungi along with various lines of morphological and cytological evidence. The smut distributions would seem to confirm the apparent close relationship between the sections of the Vesicariae complex, although they do not provide any definite grounds for treating these as a single section. However, it must be admitted that some of the arguments which Saville & Calder put forward concerning the phylogeny of Carex are somewhat dubious. In particular, it is difficult to accept their contention that 'specialised, climax groups' such as Uncinia could not have given rise to further evolutionary developments, including C.microglochin and the Primocarex species which possess a rachilla. It is also hard to accept Saville & Calder's assurance that the smut species are 'essentially natural' as they are based on morphological data only. While the evolutionary development of the smuts may have been from relatively smooth-spored forms to warty forms as the authors suggest, it seems likely that undetected reversals of such evolutionary trends would be more common in the fungi than in a group with a more complex structure which would tend to preserve more traces of the evolutionary history of the organisms. In spite of these objections, the data from parasite distributions, does seem to agree, in part at least, with morphologically based classification schemes. The validity of the observed host preferences might possibly be confirmed by experimentally induced infections and this would be of particular interest if a group of fungal species which had been investigated by biosystematic methods could be used. As Davis & Heywood (1963) point out, it is difficult to reach any firm conclusions on host phylogeny if the phylogeny of the parasite is not known with a reasonable degree of certainty.

In view of the uncertainty surrounding the sectional limits of the Vesicariae complex, it was decided to examine the anatomy, biosystematic relationships and seed protein composition of the British species in the complex in an attempt to provide a more satisfactory basis for the delimitation of sections.

2.2 The British members of Sect.Vesicariae

Four species belonging to this section are at present known to occur in Britain - C.rostrata, C.vesicaria, C.saxatilis and C.grahamii (Clapham, Tutin & Warburg, 1962), but Jermy & Tutin (1968) recognise only three, considering that C.grahamii is of hybrid origin and is not a distinct species. All of these species are extremely variable, both within Britain and in their extra-British distribution. Reports of hybrids are frequent and hybridisation may be one of the factors responsible for part of the variation range observed. On a world basis, the taxonomy of the first three species is extremely complex, with large numbers of rather dubiously separable segregate species having been recognised in different parts of their ranges.

A key to the British taxa is given in Appendix 3.

Habitat descriptions given below refer to Britain only.

1) C.rostrata Stokes in With., Arrang.Brit.Pl. ed. 2 1059 (1787)

Syn: C.vesicaria var. β L., Sp. Pl. ed. 1, 979 (1753)
C.inflata Hudson p.p., Fl.Angl. ed. 1, 354 (1762)
C.ampullacea Goodenough, in Trans.Linn.Soc.2: 207 (1794)
C.inflata Suter, Fl.Helvetica ed. 1, 2: 265 (1802)

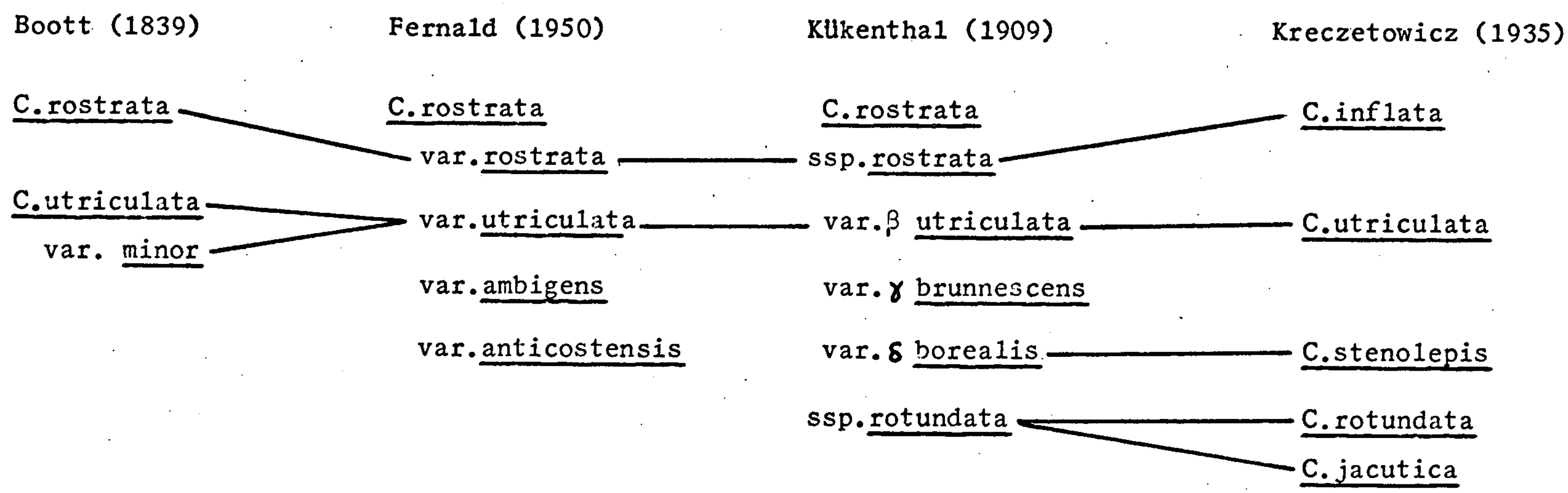
A tall perennial, up to c.100 cm. high, with long creeping rhizomes. Stem ± trigonous, rounded below; leaves up to 70 (-120) cm.x(2-)3-5(-7)mm. slightly glaucous above, somewhat inrolled or slightly keeled; ligule rounded to truncate or emarginate, up to 5(-7) mm. long, lower leaf sheaths spongy in texture. Male spikes 2-4, lower with setaceous bracts, (1-) 2-8 cm. long; male glumes 4-6 (-7.5) mm. x 0.7-1.3 mm., obovate to oblanceolate, obtuse or acute. Female spikes 1-4, erect or rarely slightly pendulous, (3-) 4-8 (-10) cm. x 6-9 mm., lower bract leaf like, from slightly shorter than the inflorescence to distinctly longer, female glumes (2-) 3-5.5 mm. x 0.8-1.2 mm., lanceolate to oblanceolate, ± acute. Utricles 3.5-6.5 mm. x 1.0-2.5 mm., ovoid, inflated, rather abruptly contracted into a smooth beak 0.7-1.9 mm. long, bifid, length of teeth variable. Nutlet subglobose-trigonous, up to 2 mm. Stigmas 3.

A plant of wet, marshland areas, usually in mesotrophic habitats on the edges of lakes, ponds and slow-moving rivers, often forming a distinct pioneering zone and extending for some distance into the water; occasionally in more acid habitats. From sea level to c.1,000 m. but rare on mountains. Occurs throughout Britain but is commonest in Scotland and N.E. England, becoming very local in the South of England. It is a circumpolar species, occurring around the Northern Hemisphere in a broad belt from just North of the Arctic Circle to c.40°N. (Europe). It extends into N.Spain, Italy and Greece and also occurs in the Caucasus. In Asia, it is present in most of N.Siberia and reaches Kamchatka, N.China and Japan in the East. There are also reports of this species from the Western Himalayas (cf. Hulten, 1962). In North America, the main distribution area is in the South of Canada and Northern United States, but the species occurs in most of Northern mainland Canada and Alaska and is present at higher altitudes in the Rocky Mountains as far South as the Mexican border. It is also known from S.Greenland and Iceland. (cf. map 95 in Hulten, 1962).

Carex rostrata is extremely variable and has often been split into a number of segregate species. Kükenthal divided it into two subspecies - rostrata and rotundata, further subdividing ssp. rostrata into four varieties. Scandinavian and N. American authors at present treat ssp. rotundata as a distinct species (C.rotundata Wahl.) and this appears to be generally accepted. Kreczetowicz (1935) also maintains C.rotundata and in addition, recognised C.stenolepis Less. (= Kükenthal's var. borealis Hartm.), C.utriculata Boott (= var.utriculata (Boott) Bailey) and C.jacutica Krecz. (= ssp.rotundata ex min. parte) as well as C.inflata Huds. (= C.rostrata Stokes). In North America, Kükenthal's var.utriculata was considered by Francis Boott to be a separate species (C.utriculata Boott) in his treatment of Carex for Hooker's Flora (Boott, 1839) and he believed that typical C.rostrata of the European type only occurred in the extreme north of the American continent. Subsequent authors treated the plant as a variety or did not accord it any formal recognition. Fernald (1942, 1950) divides the N.American material of C.rostrata into four varieties - rostrata (European type), utriculata, anticonstensis and ambiguens, but considered the last two to be rather doubtful and to require further study. A summary of these classifications is given in Fig.2.4.

C.rostrata is also known to be highly variable on a more local scale, with well-marked differences between and within populations, especially in utricle size and shape, glume size and shape and the general size and habit of the plants (cf. Jermy & Tutin, op.cit). In a number of sites in the West of Ireland, a very large broad-leaved form occurs and was recorded as C.laevirostris Blytt ex Blytt & Fries (= C.rhyncophysa C.A. Mey.) by Praeger (1893), but it was later realised that these plants were not identical with Scandinavian material of this species (Druce, 1899) and the Irish material was therefore included in C.rostrata.

Fig.2.4 Summary of treatments of *C. rostrata* s.l.


Boott (1839)
Fernald (1950)
Kükenthal (1909)
Kreczetowicz (1935)
C. rostrata
C. utriculata
var. minor
C. rostrata
var. rostrata
var. utriculata
var. ambigens
var. anticostensis
C. rostrata
ssp. rostrata
var. β utriculata
var. γ brunnescens
var. δ borealis
ssp. rotundata
C. inflata
C. utriculata
C. stenolepis
C. rotundata
C. jacutica

<u>C.rostrata</u>

Chromosome numbers 2n = 60, 72-74, 76, 82.

Reported hybrids:

x *vesicaria* (*C.* x *involuta* (Bab.) Syme. = *C.* x *pannewitsiana* Fig.)

x *saxatilis*

x *acutiformis* (*C.* x *beckmannia* Fig.)

x *pseudocyperus* (*C.* x *schmidtiana* Junge)

x *riparia*

x *hirta*

x *lasiocarpa*

x *laevirostris*

2) *C.vesicaria* L., *Sp.Pl.* ed 1, 979 (1753)

Syn: *C.inflata* Hudson, *Fl.Angl.* ed. 1, 354 (1762), non Suter (1802)

A tall perennial, up to 100 cm. or more with creeping rhizomes (usually shorter than in *C.rostrata*). Stems distinctly trigonous; leaves up to 60 (-120?) cm. (3.5-) 4-5.5 (-8) mm. green or dark green, plicate tapering into a ± flat point; ligules acute or occasionally rounded, (2.5-) 3.5-8 (-10) mm. long, lower leaf sheath not spongy. Male spikes 2-4, lower with setaceous bracts, (1-) 2-4.5 cm. long; male glumes 5-7.5 (-8.5) x 1.0-1.6 mm., oblanceolate, acute or obtuse. Female spikes 1-3, erect to somewhat pendulous, (1-) 3-5 (-6) cm. 9-15 mm., lower bract leaf-like, usually exceeding the inflorescence; female glumes 3.5-6.0 (-7) x 0.8-1.3 mm., lanceolate-oblong, acute. Utricle 5.5-8.5 (-9) x 1.5-2.5 mm., ovoid-ellipsoid, inflated, gradually contracted into a short smooth beak 0.8-1.6 mm. long, bifid. Nutlet obovoid, trigonous, up to 2 mm. Stigmas 3.

A plant of wet and marshy habitats, usually on more base-rich substrates than C.rostrata. Like the latter, it is often found on the edges of lakes, ponds and slow moving rivers but usually forms a zone behind C.rostrata if the latter is present. C.vesicaria is frequently found in wet scrub or fen woodland - a habitat in which C.rostrata very rarely occurs - and generally appears to be more tolerant of shading. It does not seem to be found on high mountains and I have not seen it at the altitudes which C.rostrata occasionally reaches (c. 1,000 m.). The species occurs throughout Britain, but is more local than the previous species and is almost completely absent from the N.W. of Scotland. On a world scale, the distribution of C.vesicaria is similar to that of C.rostrata but it is generally slightly more southern in Eurasia and the rest of its distribution is more disjunct. It reaches N. Siberia but does not extend so far north as C.rostrata. In the East, it is present in Japan and Kamchatka and extends further S.W. in China. A segregate species, C.pamirensis C.B. Clarke, occurs in the Pamirs. In N.America, C.vesicaria is much less widespread than C.rostrata s.l., being absent from Alaska and N.W. Canada and not reaching as far north as the Hudson Bay coast. Hulten (op.cit.) maps five varieties in N.America, with a gap in the mid-west where the species does not occur, and a rather disjunct Western distribution with two main centres - a) Southern British Columbia, Washington, Oregon and California; b) Colorado, Utah and Wyoming. The Eastern part of the range is occupied by a complex of more or less distinct varieties from Labrador and Newfoundland South to Tennesee. C.vesicaria does not occur in Greenland or Iceland.

The variation range in C.vesicaria, like that of the previous species, is very extensive. Kükenthal described five varieties in addition to the type (excluding vars. *grahamii* and *alpigena* and ssp. *saxatilis* dealt with below). Kreczetowicz separated *C.vesicata* Meinsh. (= var. *tenuistachya* Kük.) on the basis of its smaller size. Fernald (1950) recognises six varieties in N.America, two of which have been raised to species level by various authors. Like *C.rostrata*, *C.vesicaria* is very variable on a small scale, although this is not usually quite so noticeable as in the former species. Plants in Scandinavia and other parts of Europe frequently have shorter, wider utricles than typical British material but the differences are probably not worthy of formal recognition.

Chromosome numbers 2n = 74, 82, 86.

Reported hybrids:

x *saxatilis*

x *riparia* (*C.* x *csomadensis* Simonk.)

x *hirta* (*C.* x *grossii* Fiek).

x *lasiocarpa*

x *pseudocyperus*

3) *C.saxatilis* L., *Sp.Pl.*, ed. 1, 976 (1753)

Syn: *C.pulla* Goodenough, in *Trans.Linn.Soc.* *3*: 78 t. 14 (1797)

C.vesicaria ssp. *saxatilis* (L.) Hooker f., *Stud.Fl.Brit. Islands*, 421 (1870).

A small perennial, up to 30 or 40 cm., with a creeping rhizome. Stems ± trigonous; leaves up to 15 (-30) cm. 1.5-3.0 (-4.0) mm., green or dark green channelled to slightly keeled, with a long trigonous tip; ligule rounded, 0.5-2.5 (-4) mm. long. Male spike 1 (-2), 0.5-1.5 cm. long, bracts glume-like; male glumes 3.0-5.0 x 1.0-2.0 mm., obovate to oblanceolate, ± obtuse. Female spikes (0-) 1-2 (-3) erect and ± sessile, lower sometimes somewhat pedunculate, 0.7-2 cm. x 5-8 mm.

$\pm$ ovoid, lower bract leaf-like, usually shorter than the inflorescence, sometimes very short and setaceous; female glumes 2.0-3.5 x 1.0-2.0 mm., ovate to obovate, purple-brown, apex acute to obtuse. Utricles 2.0-4.5 x 1.2-2.0 mm., ovoid, inflated, shiny and nerveless, tapering into a very short smooth beak, up to 0.5 mm. long, $\pm$ truncate or notched. Nutlet subglobose, biconvex. Stigmas usually 2.

In Britain, C.saxatilis is a mountain species, usually occurring above 800 m. (2,500 feet) in mires and flushes (though not common in extremely base-rich habitats), especially on gently sloping saddles between mountain tops. It is found only in Scotland, mainly on the higher mountains to the west in Perthshire, Argyll and Invernesshire, but occurs sporadically in the Cairngorms and Glen Clova area. This species has a markedly Northern circumpolar distribution, being a common component of tundra and high mountain vegetation. Hulten (1962, map 16) divides the species into two subspecies and includes the segregates known in N.W. America and E.Asia as C.physocarpa. Defined in this way, C.saxatilis occurs around the Northern coasts and mountains of Europe, Siberia (incl. Kamchatka) and the whole of the extreme North in North America. It also occurs around the coasts of Greenland and in Iceland. There is a major Southern extension of its range S.W. from E.Siberia into N.China and Mongolia. In N. America it extends South along the Rockies to Utah.

The variation pattern in C.saxatilis is extremely complex. Kükenthal regarded this plant as a subspecies of C.vesicaria L. (probably because of the existence of intermediates in the form of C.grahamii and other varieties) but subsequent authors have treated it as a distinct species. It is very variable, ranging from a small plant with sessile or shortly peduncled, erect, ovoid female spikes to much taller forms with long peduncled, drooping female spikes. The shape and degree of inflation of the utricles also varies considerably. As a result, there has been a tendency to split this species into a number of separate taxa, usually at species level, with many of them rather ill-defined and difficult to separate completely from C.saxatilis. Kükenthal recognised two varieties - var.physocarpa (Presl.) Kük. and var.compacta (R.Br.) Dew. Kreczetowicz raised the latter to species level as C.membranacea Hook. and also raised Kükenthal's var.saxatilis f. laxa Trautv. to specific rank as C.procerula Krecz. In North America, according to Fernald, two varieties (var.rhomalea Fern. and var.miliaris (Michx.) Bailey) occur in addition to C.saxatilis s.s. but some authors have preferred to maintain var. miliaris as a separate species (e.g. MacKenzie, 1935). MacKenzie also maintained C.physocarpa Presl as a distinct species as did a number of other authors, but Hulten (1962) includes this plant and a number of other forms in var.laxa Kalela and claims that it is impossible to separate C.physocarpa since this form represents only one end of a variation range without any obvious breaks between the extremes. MacKenzie and Hulten also recognised C.membranacea Hook.

Small-scale variation is widespread and there are a number of varieties and forms based on comparatively minor variation in the inforescence and in the glume and utricle shape. Some plants from Britain and N.Scandinavia somewhat resemble C.membranacea in their rather swollen inflated utricles and thick female spikes.

Chromosome number 2n = 80 (C.miliaris 2n = 40; C.physocarpa 2n = c. 80,c. 78,60).

Reported hybrids:

x rostrata

x vesicaria

4) C.grahamii Boott, in Trans.Linn.Soc. 19: 215 (1845)

Syn: C.saxatilis var. grahamii (Boott) Hooker & Arnott, Brit.Fl., ed. 8, 510 (1860).

C.vesicaria var. grahamii (Boott) Hooker f., Stud.Fl.Brit. Islands, 420 (1870).

C. x ewingii Marshall, in J.Bot. 49: 197 (1911).

C.stenolepis auct., non Lessing (1831)

A perennial, up to 40 cm., with long creeping rhizomes, stems trigonous, leaves up to 25 (-35) cm. 2.0-3.5 (-4.5) mm., green to dark green, keeled to slightly plicate, with a trigonous tip; ligule rounded, 1-3 mm. long. Male spikes 1-2, 1-3 cm. long; male glumes 3.5-7.0 x 1.0-1.8 mm., obovate to oblanceolate, acute or obtuse. Female spikes 1-2 (-3), erect, lower with a short peduncle, 1-3 cm. x 6-12 mm., lower bract leaf-like, equalling the inflorescence; female glumes 2.5-4.0 x 0.9-1.5 mm., ovate-lanceolate, acute or obtuse. Utricles 3.5-5.5 (-6.5) x 1.6-2.7 mm., ovoid-ellipsoid, inflated, gradually contracted into a short smooth beak 0.4-1.2 mm. long, ± notched to bifid. ? always sterile. Stigmas 2 or 3.

A very rare mountain species, usually on wet flushed areas on steep hillsides or in small gullies - mainly on mesotrophic sites. It is recorded for 7 mountains in Scotland, in Argyllshire, Perthshire and Angus (cf. Perring and Walters, 1962; Wallace, 1940). The extra-British distribution is rather limited, with C.grahamii and grahamii-like plants recorded only from Scotland, Scandinavia and Russia (Kola Peninsula). This species was originally described from material collected in Glen Clova in Scotland, and was apparently first discovered in 1832 by a party from Edinburgh, led by Graham (Boott, 1845). Its status has since been a matter of some controversy, with some authors preferring to maintain it as a distinct species while others regard it as being merely a minor variant of C.saxatilis. A hybrid origin has also been suggested (cf. Jermy & Tutin, 1968) with C.saxatilis and C.vesicaria as the parental species. The plant which is usually referred to as C.stenolepis in Scandinavia appears to be very similar to C.grahamii but the name C.stenolepis Less. refers to a plant which Kükenthal and Kreczetowicz both regard as being a variant of C.rostrata and this name therefore does not appear to be applicable to the C.grahamii form. I have not seen the type of C.stenolepis Less. The plants referred to under C.grahamii by Fernald (1901) are now generally considered to be distinct and the name C.x mainensis is applied to these forms. According to Fernald (1950), they are hybrids between C.saxatilis s.l. and C.vesicaria or C.rostrata. I have not seen material of this taxon.

No detailed study of C.grahamii has previously been carried out, either in Britain or Europe, and little is known of the overall variation range. Raven & Walters (1956) note that there is some variation in morphology between plants from Glen Clova and those from Perthshire. This species appears to be completely sterile under natural conditions and spreads entirely vegetatively in at least two of its localities - Glen Clova and Meall nan Tarmachan (own observations, cf. also Raven & Walters op.cit.) and seems to consist of a single clone.

I have found no reports of chromosome numbers or hybrids for this species.

2.3 Related species occurring in Europe

Two other species of Section Vesicariae occur in Scandinavia and other parts of Europe - C.rhyncophysa and C.rotundata.

C.laevirostris Fr. (= C.rhyncophysa C.A. Meyer) is a tall, lowland species, similar to C.rostrata but generally much larger, with broad leaves up to 12 mm. wide, and with large, densely packed female spikes. This species occurs in Fennoscandia, Russia, Siberia and Mongolia and may also occur in Alaska.

Chromosome numbers 2n = 74, 80, 82.

Reported hybrids:

x vesicaria

x rostrata

x hirta

C.rotundata Wahl. is a smaller upland plant which in some of its characters is intermediate between C.rostrata and C.saxatilis. The leaves are rather long and narrow frequently inrolled and the utricle is similar to that of the latter species in shape and colour but has a longer beak. The female glumes are very wide and obtuse and broadly ovate in shape (ovate to narrowly ovate in C.saxatilis). It occurs in Fennoscandia, N.Russia & Siberia and also in Alaska and N. Canada.

I have found no reports of chromosome numbers or hybrids.

CHAPTER 3 COLLECTION AND CULTIVATION

3.1 Introduction

Live plants for cultivation and population samples were collected in various localities, mainly in Britain, and seed samples were obtained both from the wild and from Botanic gardens. Details of collection sites and seed sources are given in Appendix 1. The herbaria at Edinburgh (E), Kew (K) and the British Museum (BM) were also consulted.

3.2 Collection of plants

Material for population samples was collected by choosing an area where the species desired was fairly common and then sampling at intervals of 2 - 5 m. apart, to avoid taking several plants from one small clone in a single collection area (cf. however, discussion on clones in Ch. 6). The dangers of genotype reduplication in sampling have been pointed out by Harberd (1961, 1962). In the case of the *C.grahamii* populations, the distance between samples was much less, but because these appear to consist of a single clone covering a very small area, it is impossible to sample a number of clones over a wide area as in the other species. Plants were cut at the base of the culm, removing the leaves and inflorescence but leaving the rhizome intact for further growth. This was especially important for *C.grahamii* where the populations are very small, and which in the Clova site, has already suffered heavy collecting since its discovery. A number of sample sites were used, but the area around Loch Tay was found to be especially convenient since all four species occur within a few miles of the village of Killin. Threipmuir reservoir, near Balerno, Midlothian, was also found to be very convenient for detailed observations on *C.rostrata* since this species covers a fairly large area on the northern shore of the reservoir. The latter population was easily accessible in the autumn of 1972 when exceptionally dry weather conditions made it possible to sample right to the edge of the C.rostrata zone because of the low water level. Most of the population samples consisted of 30-40 individuals but the exact number varied with the abundance of plants in any particular site. Population samples were then pressed and dried and stored as ordinary herbarium specimens.

Living material was usually collected at the same sites as population samples, and consisted of a few shoots and a short piece of rhizome together with some of the original soil surrounding the plant. It was found that these samples would stay alive for at least a week, if kept in sealed polythene bags.

3.3 Cultivation techniques

Plants for cytological observations and crossing experiments were grown in a heated greenhouse. Plastic pots were used and a mixture of potting compost, peat and sand in the ratio 2:1:1 was found to give good results with most of the species grown. I found that plants had to be repotted frequently to maintain fast growth since they soon formed an extensive mass of roots and grew very slowly unless fresh soil was available. The plants were kept moist, but not waterlogged, and it did not seem necessary to stand them in water or take any special measures to ensure an abundant supply of water. A few plants of C.rostrata were grown in a garden bed and flowered successfully but did not seem to spread vigorously. A cold period was necessary to produce flowering shoots in most of the material. This was also noted by Faulkner (1970) for species in Sect. Acutae. The plants were placed in an unheated cold-frame from November to January and brought back into the greenhouse at the end of January, but it was very difficult to ensure consistent flowering, and for this reason, I was unable to carry out a number of the crosses which had originally been planned. C.lasiocarpa did not flower at all during the two years in which I had this species in cultivation. C.saxatilis did not grow well under these conditions and most of the plants died after a few months, after seed set in the crossing experiments, and eventually it was necessary to collect fresh material for each set of crosses. Conolly & Dahl (1970) claim that C.saxatilis may be one of the species whose distribution is limited by a maximum summer temperature of 21°C. If this is correct, it may be necessary to grow this species under relatively cool summer conditions for healthy growth. The greenhouse in which my specimens were grown certainly reached temperatures well above 21°C. in summer. It is interesting that C.grahamii nevertheless survived and grew in the greenhouse, although it was perhaps not quite so vigorous as some of the other species grown. C.rostrata was also grown successfully using a sand culture technique in which plants were grown in sand in polythene lined pots which were topped up with diluted Hoagland's solution (formula in Appendix 2) so that they were constantly immersed in dilute nutrient solution. These conditions produced continuous vigorous vegetative growth.

The only pests which occurred were various aphids, on the young leaves and growing points and occasionally on the roots. Those which attacked the leaves were difficult to control since they penetrated down between the older leaf sheaths, causing considerable damage to the young emerging leaves, and were protected from sprays. Fumigation with 'Fosferno' (a parathion compound) was found to be effective and completely eliminated these aphids. Root aphids were controlled by watering with a solution of 'Gammalin 20' (a BHC compound) which was very effective. Seedlings were occasionally attacked by small slugs but this was not a problem with larger plants.

3.4 Collection, germination and storage of seeds.

Seeds were collected at a number of sites, listed in Appendix 1. These were air dried and stored at room temperature in envelopes. Certain samples were obtained from botanic gardens which are also listed in Appendix 1.

Although most of the seed samples were used for protein studies, a number of germination tests were carried out to check the germination conditions required for seeds produced from crossing experiments. The first of these tests applied conditions described by Faulkner (1970) as being suitable for seeds of sect. *Acutae*. These were:

1. empty utricles discarded and only those containing nutlets retained;
2. planted in John Innes no.1 compost and kept moist;
3. a constant temperature of 20°C;
4. a 16 hr day at c. 1,200 foot candles.

Faulkner found that all the members of sect. *Acutae* which he tested under these conditions gave high germination rates. In two species of this section which I tested (*C.nigra* and *C.aquatilis*) this was confirmed and quite high germination rates obtained. Several other species also germinated quite well but *C.rostrata* gave a rather low percentage (c. 15%) and a subsequent test for this species gave no germination at all during a three month period. No germination was obtained with *C.saxatilis* and a number of other species and I therefore decided to investigate the germination requirements of these species rather more closely. *C.rostrata* was used for these experiments since a large collection of seeds from a small area at Threipmuir was available, giving more or less uniform material for tests. In all of these, a seed was scored as having germinated if both radicle and plumule had emerged.

3.4.1. Germination requirements in C.rostrata

A number of experiments on the germination requirements of this species were carried out by Thomson (1962, unpublished). Thomson's treatments included variations of lighting, temperature (constant and fluctuating), water level, water type, chilling, freezing and surgical treatments of the seeds. In spite of this wide range of treatments, he obtained no germination in the majority of his tests, and in those which did produce some seedlings, the maximum germination percentage appeared to be c.15% (3 out of 20). Of all the experimental techniques used, only freezing entire seeds for a two week period in a deep freeze or decoating and chilling the seeds for two weeks at 2-4°C. gave any germination (in both cases the seeds were subsequently subjected to an alternating 19-11°C temperature cycle).

It was intially decided to carry out a small series of experimental treatments, planting the seeds out in constant temperature conditions as used by Faulkner. A 1% water agar gel was used as a substrate (cf. Thompson & Brown, 1972) since this was clean, easy to use and allowed extensive root development and is easily cut out to remove young plants. Peat was used in some earlier tests but root formation seemed to be strongly inhibited by this material and there was some difficulty in growing young plants that resulted. This may be of some importance in determining the substrates on which C.rostrata seedlings can become established in the wild, but requires investigation in greater detail. A combination of the following treatments was used:-

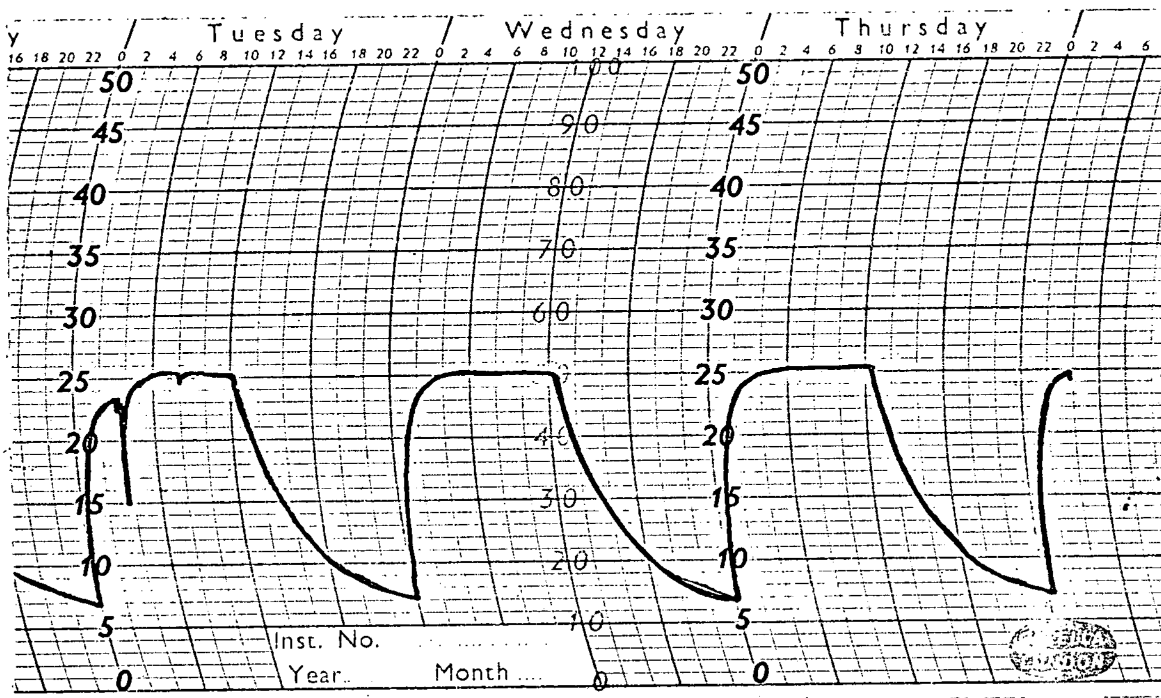
1. entire fruits with the utricles left intact;

2. utricles removed;

3. utricles removed and nutlets scarified by immersion in 50% H_2SO_4 for 3 hrs.

In treatment 1, only utricles containing nutlets were used. These can be detected by pressing each utricle with a finger tip and if a nutlet is present, it can easily be felt through the utricle wall. Fruits from each treatment were imbibed in distilled water for 24 hrs., split into two batches of 200 seeds each and one batch planted out directly while the other was chilled for 12 days at 3-4°C. and then planted out. All the seeds were placed in a growth room under the same conditions as used by Faulkner. After four months, no germination had occurred although the seeds still looked healthy, were still fully imbibed and the endosperm and embryo appeared to be undamaged. At this point, I decided to test the effect of fluctuating temperatures, since this treatment had been found to be useful in promoting the germination of C.pendula by Thompson & Brown (op.cit.). Since each group of 200 seeds had been planted in two separate petri dishes, it was possible to place 100 seeds of each treatment combination in a fluctuating temperature environment, leaving the remaining 100 in constant temperatures as a control. Fluctuating temperatures were obtained by placing an incubator in a cold room at 4°C. with the temperature control on the incubator set to give 23°C. but with a time control giving a 12 hrs on/ 12 hrs off cycle. This produced a regularly alternating temperature cycle from 23°C. maximum to a 5°C. minimum, of the form shown in fig.3.1. After two weeks of this treatment, germination began while the control showed no change. After a further two weeks, the seeds in the control batches were also placed in the fluctuating temperature environment and germination again occurred after about two weeks. The final germ-

Fig. 3.1 Temperature cycle used for seed germination in C.rostrata.



Tuesday
Wednesday
Thursday
Inst. No.
Year
Month

mination percentages obtained after a further month of treatment are shown in Table 3.1 where it can be seen that the maximum germination percentage was about 50%. This is not very high, but is a considerable improvement on both my own previous results and those of Thomson. It is possible that the long period spent at constant temperatures while the seeds were fully imbibed may be responsible for some loss of viability.

Table 3.1 Results of germination tests - effects of removal of utricles, scarification and chilling. Figures indicate % germination.

	utricles intact	nutlets only	scarified nutlets
unchilled	20	16	22
chilled	16	51	27

From the results given in Table 3.1 it would seem that the highest germination percentages are given by a combination of removal of the utricle, chilling for two weeks at 2-4°C. and a fluctuating temperature cycle.

A further test was carried out at the beginning of 1974 when the seeds were 18 months old. In this case, nutlets with the utricles removed were split into two batches:

A. nutlets untreated;

B. nutlets scarified by immersion in 75% H_2SO_4 for 20 minutes.

Each of these was further subdivided into:

1. nutlets imbibed 24 hrs at room temperature;

2. nutlets imbibed 24 hrs at 2-4°C.

A shorter soaking time for scarification was used to avoid possible damage by acid penetrating the seed coat.

Sets of 50 nutlets from each treatment combination were planted out on 1% water agar as before. A second set of nutlets was planted out after four days of treatments 1 and 2. All the seeds were placed in the fluctuating temperature conditions described for the previous experiment. Germination occurred after a period of 10 - 12 days and the results are summarised in fig. 3.2. It can be seen that scarified seeds germinated more quickly and gave a greater germination percentage than unscarified seeds. In addition, chilling appeared to produce no improvement in germination, and, if it had any effect at all tended to slow down the germination rate.

Another small scale test was carried out to give some indication of whether the utricle contains any soluble inhibitor since germination appeared to be considerably reduced if the utricle is left intact. A batch of c.200 complete fruits with utricles was imbibed for 3 days in a small volume (c. 10 ml.) of tap water. A second batch was washed for 3 days in a continuous flow of tap water, in an attempt to wash out any soluble inhibitor which might be present. 25 fruits from each treatment were planted out as in previous experiments. As a control, batches of 25 nutlets without utricles were planted out on 1% water agar and 1% agar made up with 1/5 normal strength Hoagland's solution (to check the effects of nutrient level on germination and the growth of the young seedlings). The results are shown in Table 3.2 below:

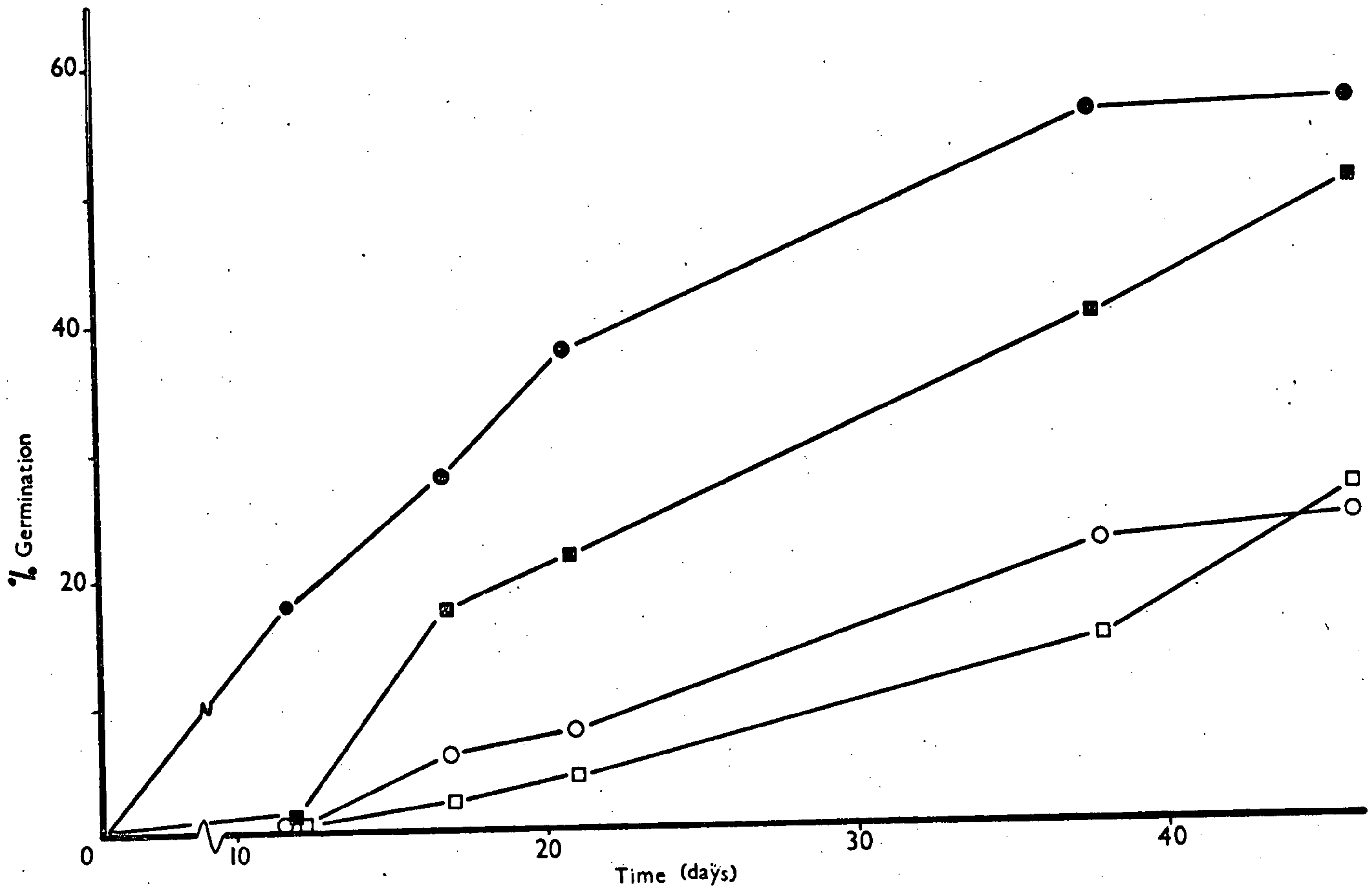
Fig. 3.2 Germination of *Carex rostrata* seeds under different experimental conditions.

(●) scarified and imbibed at 20°C.

(○) unscarified and imbibed at 20°C.

(▣) scarified and imbibed at 4°C.

(□) unscarified and imbibed at 4°C.



60
40
20
0
% Germination
10
20
30
40
Time (days)

Table 3.2. Results of tests on washing and increased nutrient levels.

treatment	germinated seedlings	%age germination
1. utricles intact, unwashed	1	4
2. utricles intact, washed for 3 days	2	8
3. nutlets only, on water agar	13	52
4. nutlets only, on Hoagland agar	13	52

It is obvious from the above that a) washing of the utricles for 3 days does not produce a significant increase in germination, b) removal of the utricles does give a considerably improved germination percentage,(c. 50% as opposed to 8%), and c) there is no improvement in germination rate when extra nutrients are added to the agar. In addition, there was no obvious increase in size or vigour in the seedlings grown on nutrient-rich agar. It is possible that the effect of the utricle is mainly mechanical since it was noted that in some of the fruits with intact utricles, the plumule emerged from the nutlet but appeared to be unable to escape from the confining utricle, becoming coiled up inside and eventually dying without emerging. An alternative explanation might be that a rather insoluble inhibitor is present which is not removed by a 3-day wash but requires a much longer period. This may not be entirely unreasonable since in the natural habitat <u>C.rostrata</u> fruits would probably be surrounded by large volumes of water for several months, allowing gradual slow diffusion, but if this is the case, it may be rather difficult to separate the effects of bacterial and fungal attack on the mechanical strength of the utricle and a slow leaching process without a much more elaborate experimental design.

From the results of the experiments described above, it would appear that there are two major requirements for good germination of C.rostrata seeds under laboratory conditions:

1) A fluctuating temperature cycle;

2) Removal of the utricle.

The first is almost essential since little or no germination takes place at constant temperature (although these experiments do not rule out the possibility that germination might be improved at constant temperatures above or below 20°C.). The second condition is slightly less critical since some germination does occur if the utricles are left intact. Two other factors which may improve germination are (3) chilling and (4) scarification, but the evidence for these is more contradictory. Chilling of the imbibed seeds in the first experiment appeared to increase the germination percentage but in the second, it seemed to have the opposite effect, lowering the number of seeds germinating and slowing the growth of those which did. This may be caused by a change of requirements as the seeds age since there was a considerable difference in the age of the seed used in the two experiments, (4 months and 18 months). The evidence from hybrid seeds grown (see next section) would seem to support the existence of some form of requirement for chilling in fresh seeds, and this may be lost after prolonged storage. Scarification, in the first experiment, appeared to decrease the germination obtained but both the percentage and speed of germination were considerably improved by this treatment in the second experiment. A possible explanation lies in the different scarification treatments given - 3 hrs in 50% and 20 mins in 75% H_2SO_4. It is possible that the prolonged treatment in the first experiment damaged some of the seeds, while the shorter but more intense treatment of the second experiment removed a similar amount of material but did not penetrate the seed coat.

3.4.2. Germination in other species of Carex

I have not carried out any large-scale germination tests in other Carex species because of limitations of time and materials, but a few small tests were made on field collected seed samples, and seed obtained from hybridisation experiments (cf. Ch. 7), and these help to extend the observations for C. rostrata.

1. Hybrid seed

Seeds which had been collected from a number of different crosses (cf. Ch. 7) were stored for about 1 month and then planted out on 1% water agar in two batches. In both cases the utricles had been removed and the seeds placed in fluctuating temperatures, but the first batch was imbibed and planted directly while the second was imbibed and chilled for two weeks at 2-4^{o} before planting.

Germination in the first batch occurred after 6-8 weeks and was rather irregular, often with long intervals between germination of seeds from the same cross. Those which germinated first were from a cross in which C. pseudocyperus was the maternal parent, and these germinated fairly uniformly. Other crosses, however, took much longer and only germinated after 3-4 months. In the second batch, germination commenced 13 days after planting and was very uniform, with almost all of the viable seeds germinating at the same time. It would seem from these tests that some degree of chilling is beneficial, at least in fresh seed.

2. Othdr species

A number of species from different sections were tested under constant temperature conditions. Some of these germinated while others failed completely. Seeds which did not germinate were subsequently moved into fluctuating temperature conditions after a period of 5 months. Germination then occurred in a wider range of species and the results are summarised in table 3.3 below. Since these were usually very small samples of c.5-10 seeds, I have not used percentage figures, and have merely indicated the relative ease of germination under the different conditions used.

It is noticeable that the members of Sect.Acutae tested gave good germination under constant temperature conditions, in agreement with Faulkner's (1970) observations, with the exception of C.rufina which is a high mountain and arctic species, but even this gave some germination in constant temperatures.

3.4.3 Germination requirements and ecology in Carex

Since these experiments were carried out with the aim of finding conditions under which reasonable germination percentages could be obtained, they do not by any means provide a detailed survey of germination requirements and ecology of C.rostrata under natural conditions. In spite of these reservations, however, I feel that some useful indications of the factors involved in the germination ecology of this species are provided by the results outlined above. It would appear that the following factors are essential for or to some degree promote the germination of C.rostrata seeds:-

1. Fluctuating temperatures;
2. Removal of the utricle;
3. Chilling at 2-4^{o}C. for 2 weeks.
4. Scarification.

It is interesting to compare these with the results obtained by Thomson (op.cit.) who tested the effects of alternating temperatures and cold

Table 3.3 Germination of various Carex species.

Section	Species	constant temperature	fluctuating temperature
Spirostachyae	binervis	-	++
Capillares	capillaris	-	+
Montanae	ericetorum^{n}	-	±
Atratae	adelostoma^{n}	-	++
	norvegica^{n}	-	++
Acutae	aquatilis	++	nt.
	nigra	++	nt.
	rufina^{n}	+	+
Leucoglochin	microglochin^{n}	++	++
Microcephalae	capitata^{n}	-	++
Dioicae	parallela^{n}	-	++
Divisae	chordorrhiza^{n}	-	++
Incurvae	maritima^{n}	±	nt
Canescentes	lachenalii^{n}	+	+

++ abundant germination (ca. 40%).

\+ only a few seeds germinating (ca. 20%).

± one or two seeds only.

\- no germination over a five month period.

nt not tested

n seeds collected in Norway, otherwise all material was British treatments but obtained very little germination. A possible explanation for this failure of germination may be that the range of temperature which he used was much less than that used in this study, viz 10°C. as opposed to 18°C. Furthermore under the particular experimental procedure used to give this range, the minimum temperature was 9.5°C. and not as low as the 4-5°C. used here. Taylor (1956) claimed that a 9-12 month after-ripening period was required in C.flacca but does not give details of the conditions used so it is presumed that constant temperatures were employed. It is surprising that his is the only account of a Carex species in the 'Biological Flora of the British Isles', in view of the considerable ecological importance and interest of this large genus which is so well represented in Britain. Taylor does not appear to have tested the effects of chilling or made any sort of surgical alterations to the complete fruits with intact utricles.

The requirement for a fluctuating temperature is of considerable interest with regard to the general ecology of C.rostrata. The subject of temperature requirements is reviewed by Koller (in Koslowski, 1972), who points out that the restriction of germination to conditions where the temperature regularly fluctuates may be an important mechanism, preventing germination of small seeds which are deeply buried in soil where atmospheric temperature oscillations are damped down below the critical range required. Seeds which are deeply buried are kept at almost constant temperature and thus do not germinate. A surface temperature range of 20°C.may be reduced to 4°C. range at a soil depth of c. 30 cm (Van Wijk, 1963). This would also apply to seeds at differing depths in a pool of water, and the restriction imposed by this germination requirement may be of considerable importance in C.rostrata, allowing germination only in seeds placed near or on the water's edge or buried to a shallow depth in soil or mud. If the requirements for chilling and removal of the utricle are genuine effects, then a possible sequence of events in the life cycle of C.rostrata can be built up as follows - (1) autumn - ripening and release of utricles containing mature nutlets. (2) late autumn - fruits float for varying periods (up to 1 month or more - cf. Thomson op.cit.) and eventually sink or are washed up at the water's edge, (3) winter - fruits are chilled and decay of utricle begins, (4) spring - utricle decays further and average temperature begins to rise, (5) early summer - average temperature higher, temperature fluctuations of sufficient amplitude and maximum to promote germination, (6) summer and early autumn - establishment of seedlings. I have not seen any seedlings at any of the sites visited and since Thomson also failed to find any, it seems likely that seedling establishment is a rather rare event in this species. In those species which germinate quickly in constant temperatures, it seems likely that the conditions required for germination are rather less critical and might be present at the time when the fruits are shed, especially in the earlier flowering species. It is possible, however, that other factors not considered here may prevent early germination.

Because of the limited nature of this study, the possible effects of a number of important factors have not been considered. In any detailed study of the germination ecology of C.rostrata, the following factors would have to be investigated to provide a more extensive knowledge of the precise conditions required by this plant - light intensity and duration, minimum and maximum temperature ranges for effective germination, changes of requirements with age, the effects of wet storage and the interactions between these variations in the environment. The effects of chilling and scarification also need to be investigated in more detail. It must also be borne in mind that C.rostrata is very vigorous vegetatively and that single clones may cover quite large areas (cf. discussion on clonal structure in Ch.8). Seed production is therefore probably of minor importance in much of the spread of this species and is probably only significant in establishing the plant in new, previously uncolonised habitats. Individual clones may reach considerable ages - Harberd (1961) claims that some Festuca clones may have survived in the same area for thousands of years - and the breeding behaviour of such species may be more similar to that of long-lived trees rather than herbaceous forms with much shorter life cycles (cf. Ch.7).

Since it is fairly widespread and grows in a comparatively narrow range of habitats C.rostrata offers interesting possibilities as research material for an autecological study. It would be of interest to know something of the variation in germination requirements between different clones and between different parts of the species range, since its area is very extensive. In particular, a survey of the requirements of the N.American var. utriculata and plants from more extreme parts of the species range may reveal some interesting patterns of adaptation to local conditions. Thompson (in Heywood, 1973) has found considerable differences in germination requirements of seeds of Silene species collected in different parts of Europe, especially between Northern and Southern populations. It would also be very informative to compare the C.rostrata in detail with C.vesicaria. Although this species often grows in similar environments to C.rostrata it appears to be much more tolerant of low light levels and an examination of the effects of light on germination and seedling establishment may indicate something of the mechanism involved in the ecological separation of the two species.

In conclusion, it would appear that the set of conditions outlined at the beginning of this section is sufficient to produce reasonable levels of germination in C.rostrata. Other species of Sect.Vesicariae have not been tested in detail, but hybrid seed of C.vesicaria, C.saxatilis and C.grahamii germinated under these conditions, as did hybrid seed of members of a number of other Sections, including C.hirta and C.pseudocyperus, and it would therefore seem likely that these conditions may be applicable to a wide range of Carex species, at least in the North Temperate and arctic areas.

4.1 Introduction

The purpose of this chapter is to review previous chemotaxonomic studies on Carex and to describe the studies made on members of Sect. Vesicariae and other Carex spp. during this investigation.

The total amount of chemotaxonomic work carried out in the Cyperaceae is very small in comparison with chemically better known families such as the Leguminosae and Umbelliferae. Most of the studies so far attempted have been concerned with flavonoids and similar compounds. Clifford & Harborne (1969) investigated flavonoid pigments in a few Carex species and several more general flavonoid surveys have included species of this genus as representatives of the Cyperaceae (e.g. Harborne, 1971). These studies were intended to investigate relationships between families and higher taxonomic levels and as a result, little attention was directed towards relationships within the Cyperaceae. In general, the results suggest that there are strong resemblances between the Gramineae and the Cyperaceae in flavonoid components and that the Juncaceae are rather less closely related, but the data are as yet so incomplete that no firm conclusions can be drawn. It is interesting, however, that some strong similarities to the Palmae also exist (Harborne, 1971). More detailed studies within the Cyperaceae have been made by Kukkonen (1971), who examined 161 species for flavonoid compounds, including 24 Carex species of which C. rostrata was the only member of the Vesicariae. His results showed that these tended to have distinctive flavonoid patterns which could be used to identify species although some intraspecific variation occurred. He concluded that the Cariceae were the most homogeneous tribe in the Cyperaceae, a finding which agrees with anatomical evidence (Metcalfe,

1971) and suggests that the tribe forms a natural group of more or less closely related forms. Kukkonen does not give details of the structures of the substances extracted and did not attempt to subdivide the Cariceae on the basis of his chemical data. The most detailed studies in Carex have been confined to small sections or species groups. Toivonen (1974) examined flavonoids in Sect. Heleonastes and found patterns characteristic of the different species were present and that hybrids were usually intermediate between the parental species. The flavonoid constituents were also correlated with the morphological subdivisions in the section. Ediger (1966) examined phenolics in three species of the C. aggregata complex in the U.S.A., finding that three phenolic patterns could be distinguished, and concluded that it was possible to separate the three taxa on this basis. It is not clear, however, to what extent the differences which he observed could be explained by environmental factors, since one of the species tended to occur in drier habitats and no transplant experiments to test the validity of the phenolic differences appear to have been carried out. The dangers of possible environmental modification of phenolic spot patterns have been pointed out by Asker & Frost (1970) who found considerable intraspecific variation, and in particular, differences between old and young plants in Potentilla. Seasonal variation in phenolic constituents has also been discussed by Adams (1972). In general, it would appear that when used with appropriate controls, studies of phenolic compounds may be extremely useful in taxonomic studies in Carex as a means of distinguishing between species and identifying hybrids.

The use of chemical data at higher taxonomic levels in an evolutionary context is, however, much more difficult. Since phenolic compounds and small molecules are 'episemantic molecules' in Zuckerkandl & Pauling's (1965) terminology, they are subject to considerable uncertainty and loss of information when used as a source of characters for phylogenetic speculation. The presence of a certain substance cannot be regarded as convincing evidence for a close relationship between two taxa unless it can be shown to be synthesized by the same biochemical pathway in both. This is, of course, highly probable in two organisms which are obviously closely related on morphological and other grounds and are placed in the same taxonomic group, but where widely separated parts of the taxonomic hierarchy are being compared, the chances of the occurrence of two different pathways is much higher. Examples are given by Grisebach (1973) who points out that the naphthaquinones are known to be synthesized by at least four different pathways and includes a number of other chemical groups known to be produced by quite separate pathways. Similarly, the importance of the absence of a compound may be rather difficult to assess. It may be produced in amounts which are too small to detect in the test system used or its production may be blocked by a single mutation which may be of very little significance in phylogenetic terms. There have, of course, been numerous studies in which the use of phenolics and other small molecules have been shown to be of considerable value. The occurrence of the betalains in the Caryophyllales is a well-known example of the presence of a characteristic group of compounds within a single taxon which appears to be a well-defined natural order. Even in this case, however, there are problems of interpretation. The betalains are absent from the Caryophyllaceae, a family which appears to be central to the whole order. Considerable discussion has taken place on the exact significance of this character and whether the absence of betalains should be regarded as primitive or advanced state in the case of the Caryophyllaceae. It has even been proposed that the Caryophylales should be defined on the basis of presence of these compounds, thus excluding the Caryophyllaceae. (a review of the chemotaxonomy of the Caryophyllales and the associated problems are given by Gibbs 1974, and Fairbrothers, 1968). It is obvious that unless the investigator is prepared to study the biosynthetic pathways and chemical relationships of the compounds being used, a major undertaking in any but the simplest of systems, the use of small molecules as a source of phylogenetic information must be regarded as being of rather dubious value, and subject to considerable variation in interpretation. Studies of phenolics and small molecules in which the biosynthetic pathways are unknown are probably of maximum value at lower levels of the taxonomic hierarchy, at species or subgeneric level in comparisons of closely related species and hybrids. In these cases, phenolics and other compounds can be used in a manner similar to the treatment of standard morphological characters which have normally not been investigated at the biochemical/genetical level. Even the use of simple spot patterns of unidentified compounds can be of considerable value, as pointed out by Alston (1967).

Although the present survey was carried out at subgeneric and species group level, it was considered that a study of macromolecules would provide a more profitable source of chemotaxonomic data than the smaller molecules. The use of macromolecules is theoretically preferable for three main reasons:

1) They are semantides, i.e. they are a more or less direct expression of the genetic constitution of the organism being studied (for the purposes of this discussion, the term macromolecules refers to nucleic acids and proteins. Carbohydrates and other classes of biological polymers are episemantides).

2) They are extremely complex molecules and there is little chance of the same molecule being produced by convergence in widely differing organisms.

3) They are generally considered to be less liable to environmental modification than most morphological characters or small molecule constituents.

In view of the characteristics mentioned above, it seems reasonable to assume that macromolecular data will provide a more precise indication of genetic affinity and phylogenetic relationships than would be possible from data on small molecules.

The most readily available techniques for the study of macromolecules for taxonomic purposes are those developed for separation and identification of proteins. Two general groups of techniques have been applied in taxonomic studies because of the relative speed and the ease with which they can be utilised. These are serology and gel electrophoresis. Numerous other techniques are available for extraction and purification of proteins but these are generally applicable to studies in which specific proteins are being examined. Although data obtained from studies of the structure of single proteins may be of considerable taxonomic interest, these normally require a prolonged investigation by a skilled biochemist and are therefore not suitable for small scale preliminary studies where a mixture of proteins is being extracted. The literature of taxonomic uses of serology and gel electrophoresis is now enormous and the theoretical basis for these techniques has been discussed in a number of publications. Numerous examples of serological studies are given in Leone (1964) and Hawkes (1969) and much of the earlier work involving gel electrophoresis is reviewed by Hunziker (1969).

Virtually no work involving the use of macromolecules has been carried out in the *Cyperaceae*, the only published study being that of Lee & Fairbrothers (1968) who tested extracts from *Scirpus* against antisera prepared from *Typha* and *Sparganium*. No investigations have been made in *Carex*. It was decided initially to use serological techniques as a means of comparing seed proteins from a range of *Carex* species, with particular emphasis on members of Sect. *Vesicariae*. When it was subsequently found that *Carex* seed proteins were not entirely suitable for serological purposes, polyacrylamide gel electrophoresis (PAGE) was employed. A large number of different buffer and gel systems have been proposed and several types have been used in taxonomic studies. Since most PAGE systems separate proteins on the basis of a combination of charge and molecular weight, they tend to be sensitive to changes in the charged amino acids making up the protein molecule. A single amino acid substitution could have a marked effect on the speed at which a protein migrates in an electric field, if the substitution alters the net charge on the protein molecule. Johnson (1974) points out that even if an uncharged amino acid is replaced by another uncharged acid, an overall change in the charge of the protein may occur because the 'new' amino acid may alter the pH at which a nearby charged group becomes charged, although it does not possess any charge of its own. Small differences in protein constituents are of considerable interest in studies of population differentiation and in distinguishing closely related species but they may obscure important resemblances between more distantly related forms. It was therefore decided that a gel system containing sodium dodecyl sulphate (SDS) should be used since this system separates proteins mainly on the basis of molecular weight and charge effects are almost entirely eliminated (Laemmli, 1970; Weber & Osborn, 1969). As a result, only large scale differences involving changes in molecular weight of 10% or more (Weber & Osborn, 1969) should be detected. This system does not eliminate the problems of deciding whether or not the bands observed at similar positions on the gels are homologous, but this is a question which could only be finally settled by the use of peptide 'finger-printing' and other more sophisticated techniques. SDS gels have been applied with some success to studies of wheat proteins (Hsam & Larter, 1974).

4.2. Materials and methods

Seed proteins were used for these investigations and samples of *Carex* seeds were obtained from a number of sources (Appendix I). Nutlets with the utricles removed were ground to a fine flour in a grinding mill fitted with a 0.5 mm sieve. Weighed samples of flour were then mixed with extractant in small conical flasks to form a thick slurry and left to stand for 24 hours at 4°C. The volume of extractant varied since in some species, a large amount was absorbed by the flour and it was impossible to centrifuge a very thick slurry. The extracts were centrifuged in a bench centrifuge, filtered, and then stored at -10°C. in a refrigerator. Three main extractants were used:

1) 0.8% sodium chloride
2) 1% sodium chloride buffered to pH 8 with phosphate buffer
3) N/10 sodium hydroxide

Where extracts were intended for serological purposes, extractants 1 and 3 were used but with the latter, it was necessary to dialyse the extracts for 24 hours in several changes of distilled water to remove excess sodium hydroxide. Saline extracts could be injected directly. The protein content of the extracts was determined by the Lowry technique (Lowry et al., 1951).

Antisera were prepared in rabbits, using a standard injection schedule of two intramuscular injections of 1 ml. of antigen emulsified with Freund's adjuvant and one intramuscular injection of 1 ml. antigen solution, followed by a course of 7-9 intravenous injections of 0.5 ml antigen solution. The complete injection schedule was administered over a period of ca. 5 weeks: the rabbits were then anaesthetised and the blood for antiserum production extracted by cardiac puncture. Nembutal was used as the anaesthetic and heparin was added to prevent premature clotting. The blood collected was subsequently allowed to clot and was then centrifuged at 12,000 g. to give a clear antiserum which was stored in a plastic container at 4°C. A few drops of 10% sodium azide were added to prevent growth of micro-organisms. Serological tests were performed by the Ouchterlony double diffusion technique in a 0.6% 'Ionagar' No.2 agar gel buffered to pH 8.6 with a sodium barbital buffer. The agar was spread on 10 x 8 cm. glass plates and wells for antisera and antigens were cut with a cork borer or scalpel. The tests were run for 48 hours at 18°C. and the plates were then dried between filter papers and stained with Ponceau S.

The electrophoresis system used was basically that described by Laemmli (1970) but no stacking gel was used and 3% urea was added to the gel. A list of ingredients is given in Appendix 2. A 15% gel with a Tris/HCl buffer at pH 8.5 was cast in 10 cm. perspex tubes. The gels were prerun for 30 mins. at 1 ma./gel. and the samples were then loaded and run for 7 hours at 2 ma./gel. The samples were extracted in buffered NaCl (extractant 2) in the same way as the extracts for serology and were then made up to 1% SDS and 10% glucose before loading on the gels. A PAGE system containing high urea concentrations and no SDS was also tested. The gel concentration was again 15% but the gel buffer contained 6.25 M urea and 0.17 M acetic acid, while the running buffer was 6 M urea and 0.9M acetic acid (cf. Appendix 2). Proteins were extracted in a solution of the same composition as the running buffer, but 1% mercaptoethanol was added and after extraction and centrifugation, the protein solution was made up to 15% with sucrose to ensure that it was sufficiently dense to avoid mixing and diffusion when it was placed on top of the gel. This system is a modification of the method used by Panyim and Chalky (1969).

The gels were stained in Coomassie blue (0.25% in methanol/acetic acid/water - 45.4%:45.4%:9.2%) and destained in 7.5% acetic acid/5% methanol.

4.3 Results of serological studies

Preliminary attempts to extract albumins and globulins from Carex seeds using 0.8% saline produced very low protein concentrations (ca. 50-100 μg./ml.) which were not suitable for use as antigens. The optimum concentration for this purpose is usually around 1,000 μg/ml. or more if a reasonably small volume for injection is to be obtained. Although different species varied somewhat in the concentrations achieved, none gave a concentration which was directly usable. Some degree of concentration of the samples was possible, but this required seed samples of 50 g. or more to give adequate antigen supplies and was not feasible in most cases since only small seed samples were available. Attempts were made therefore to increase the quantities of protein extracted by altering the pH of the saline solution and by using different extractants. This is limited, however, by the requirement that the final solution must be injected into rabbits without causing any adverse effects on the health of the animal.

The effects of pH and ionicity were tested on flour of C.rostrata. Three pH levels (6.0, 7.0 & 8.0) and three levels of ionicity (I = 0.05, 0.1 & 0.2) were set up using phosphate buffers, making 9 combinations in all. 10 mls of each pH/ionicity combination were added to 1 g. samples of flour and the protein concentrations were measured after extraction for 24 hours at 20°C. in a shaking water bath followed by centrifugation in a bench centrifuge. The results obtained are shown in Table 4.1 below.

Table 4.1 Protein concentrations (μg/ml) from pH/ionicity tests

ionicity pH	6.0	7.0	8.0
0.05	0	0	60
0.1	0	15	85
0.2	0	40	90

It can be seen from this table that the general trend was an increase in protein concentration with increasing pH and ionicity, with the highest concentrations at pH 8.0 and I = 0.2. At pH 6.0, no protein was detectable in any of the samples. It is obvious, however, that the concentrations obtained were still much too low for serological purposes.

A further series of tests was carried out, using N/10 sodium hydroxide as an extractant. This produced much higher protein concentrations as measured by the Lowry test, ranging from 200 to 20,000 μg/ml, but the extracts obtained were dark brown in colour. The colouring is assumed to have been caused by tannins and polyphenolic compounds extracted from the seed coat, but their exact nature was not determined. Precipitation with TCA and dialysis against fresh extractant had no effect on the colour of the solutions, suggesting that the colouring substances were bound to the proteins. The concentrations obtained were well within the range usable for serology.

Attempts to increase the solubility of the seed proteins even more by imbibing the seeds before extraction were generally unsuccessful. Table 4.2 (below) shows the results obtained when seeds were imbibed, or imbibed and then scarified, compared with those for dry seeds.

Table 4.2 Protein concentration (μg/ml) from imbibed and dry seeds.

treatment	extractant 0.8% saline	N/10 NaOH
dry seeds	200	15,000
seeds imbibed	80	16,000
seeds scarified 30 mins. imbibed	0	16,000
seeds scarified 60 mins. imbibed	30	18,000

Scarification was carried out by soaking the seeds in 75% H_2SO_4 and a standard volume of extractant (5 mls./g.) added to the milled samples. In this test, soaking the seeds in distilled water for 24 hours markedly reduced the concentration obtained in saline and scarification of the seeds before soaking reduced the concentration even more. There is probably no significant difference between the two figures obtained for scarified samples since protein concentrations at this level are rather difficult to measure accurately. Using NaOH as an extractant however, there was practically no difference in the concentrations obtained and there was possibly even a slight increase when the seeds were scarified. The reasons for the decrease in concentration noted in the saline extracts are not obvious, but it is possible that tannins and polyphenols in the seed coat interfere with the Lowry reactions and produce spuriously high readings. When seeds are soaked or scarified, some of these may be removed and the apparent protein concentration then decreases, although the actual protein content is more or less constant. NaOH may extract fractions of these substances which are not removed, but the differences between the extractants have not been investigated in detail.

C.grayii gave the highest protein concentrations, using NaOH extraction, and solutions containing 20,000 µg./ml. were obtained. This species was therefore chosen to test the production of antisera for Carex proteins. One problem resulting from the use of NaOH as an extractant is that there is a strong possibility of the proteins being denatured when they dissolve in this solute. To test the extent of denaturation, an NaOH extract of Zea seed proteins was reacted with a Zea antiserum made from saline extracted antigens (this was kindly provided by Mr. D.G. Long). It was found that the NaOH extract did react with the antiserum, but the precise pattern of precipitin arcs formed was slightly different. At least two precipitin bands were present (Fig. 4.1). It would appear, therefore, that proteins extracted in NaOH retain enough of their antigenic properties to cross react with an antiserum made from saline extracts, and it was decided that these proteins might offer suitable material for production of a Carex antiserum.

It was not possible to use the NaOH extracts directly since the alkali content could be harmful to the rabbits, and all samples were dialysed against several changes of fresh 0.8% saline for 24 hours to remove any free NaOH present. Some protein material was

Fig. 4.1 Results of immuno-diffusion tests.

(a) *Zea* antiserum/antigen system.

S saline extract

N N/10 NaOH extract

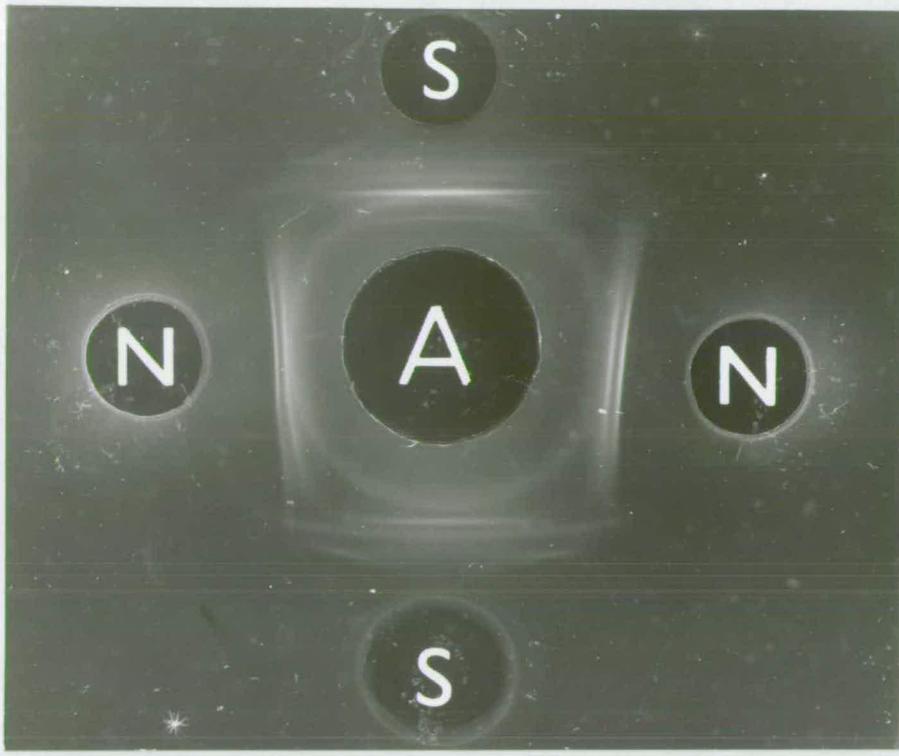
A Antiserum

(b) *Carex grayii* antiserum/antigen system

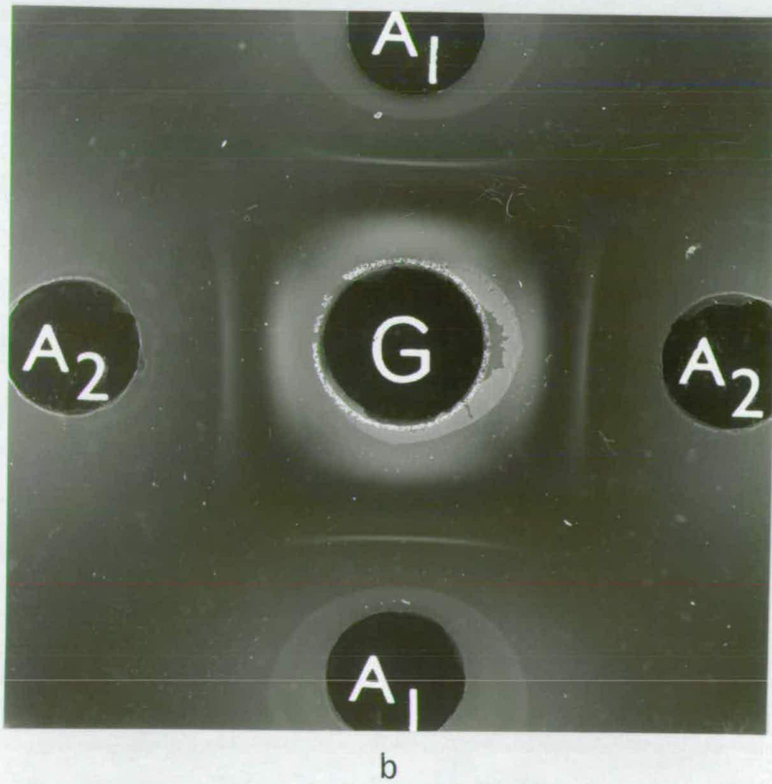
A_1 antiserum 1

A_2 antiserum 2

G N/10 NaOH extract



a



b

precipitated during this procedure, but final concentrations of 5,000-7,000 μg/ml. were obtained and were considered adequate for serology.

Four rabbits were used to produce antisera, but one of these had to be killed at an early stage because of disease. The remaining three were given a full course of 9 injections. The antisera obtained were tested against dialysed and undialysed extracts of *C.grayii* seed protein. It was found that the reactions produced were extremely weak, with only two or three faint precipitin lines in most of the tests (Fig.4.1), and the strength of the reaction was similar for all three antisera, although minor differences were present. Attempts to concentrate the antigen solution using polyethylene glycol did not produce any noticeable improvement in reaction strength. Tests made with antigens from a number of other species, including *C.rostrata*, *C.binervis* and *C.paniculata*, gave no visible reactions. Since it appeared to be impossible to produce usable results from this system and time and materials to produce another more reactive antigen system were not available, it was decided that serological investigations should be abandoned, and chemotaxonomic investigations restricted to the use of PAGE.

4.4 Gel electrophoresis studies.

Extracts for gel electrophoresis were made with buffered saline at pH 8 containing 0.75% mercaptoethanol. 12 species were examined using SDS gels and in addition, a sample of *Eriophorum angustifolium* was included to compare the extent to which protein differences might occur in different genera within the *Cyperaceae*. Tests using *C.rostrata* extracts indicated that sample loadings of 50-80 μg. of protein per gel gave optimum results in terms of the maximum number of bands resolved without excessive overloading. Similar results were given by

Fig.4.2 Banding patterns produced by _Carex_ seed proteins on SDS polyacrylamide gels.

a) _C.vesicaria_

b) _C.saxatilis_

c) _C.rostrata_

d) _C.pseudocyperus_

e) _C.aquatilis_

f) _C.binervis_

g) _C.laevigata_

h) _C.panicea_

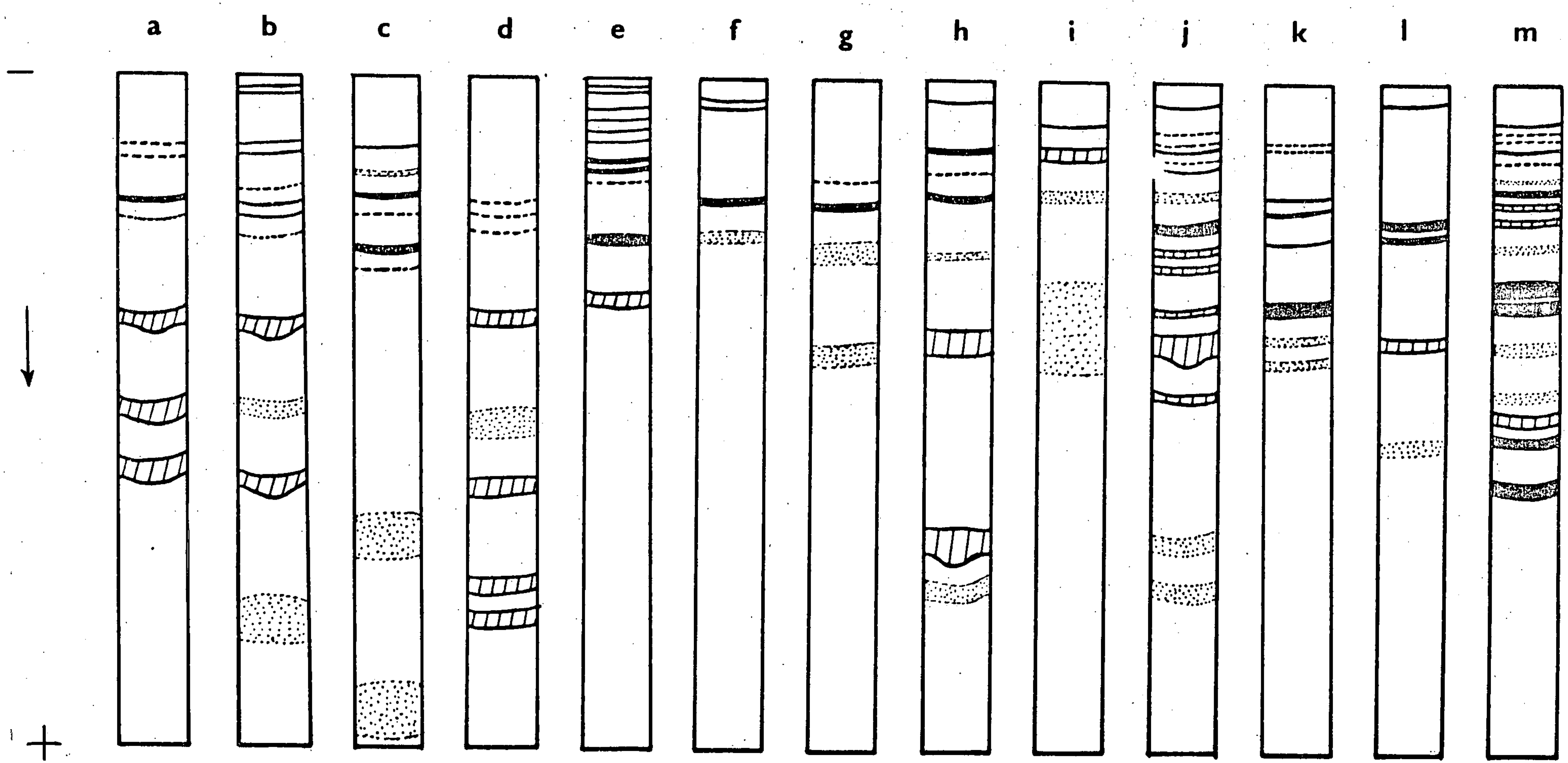
i) _C.flacca_

j) _C.pilulifera_

k) _C.davalliana_

l) _C.muricata_

m) _Eriophorum angustifolium_


a
b
c
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other species. The banding patterns produced on the gels are shown in diagrammatic form in Fig.4.2. The bands obtained from most of the *Carex* species produced only two or three bands. In marked contrast, the *Eriophorum* material produced numerous very sharply resolved bands with a characteristic reproducible pattern, and up to 25 separate bands could be distinguished on some of the gels. Only the main bands are visible in Fig. 4.3, and some of the faint minor bands have been omitted from Fig. 4.2 to avoid confusion. Since the gels obtained from *Eriophorum* extracts gave excellent results, it would appear that SDS gels are capable of producing good separation and resolution of plant seed proteins under favourable conditions. It would appear, however, that *Carex* seed proteins offer rather intractable material for protein separation techniques. The reasons for these difficulties are not known, but it is possible that substances associated with the proteins in *Carex* seeds may adversely affect the running of SDS gels. These problems are further discussed in section 4.5.

A comparison of the protein banding patterns shown in Fig.4.2 reveals a number of interesting points:-

1) The banding patterns for *C.saxatilis* and *C.vesicaria* are very similar. It is difficult to match the bands produced in tube gels because of slight variations between gels, but at least five, and possibly all seven of the *C.vesicaria* bands appear to match the pattern obtained from *C.saxatilis*. The similarity in seed protein complements of these two species therefore agrees with cytological evidence in suggesting a close genetic affinity. There are, however, some differences between them, with two high molecular weight bands present in *C.saxatilis* and absent in *C.vesicaria*, and a further pair of lower

molecular weight also absent in the latter species. This may only indicate that the corresponding proteins in C.vesicaria are present in very small amounts which cannot be detected at the gel loadings used, but even if this is so, there is a quantitative difference between the two species.

2) The pattern produced by C.rostrata is very different from those of the other two members of Sect.Vesicariae and reinforces the anatomical and cytological evidence which indicates that this species is probably rather distinct from the rest of Sect.Vesicariae.

3) C.pseudocyperus has at least three and possibly five protein bands which match reasonably closely with C.vesicaria, suggesting a close link between this species and the Vesicariae.

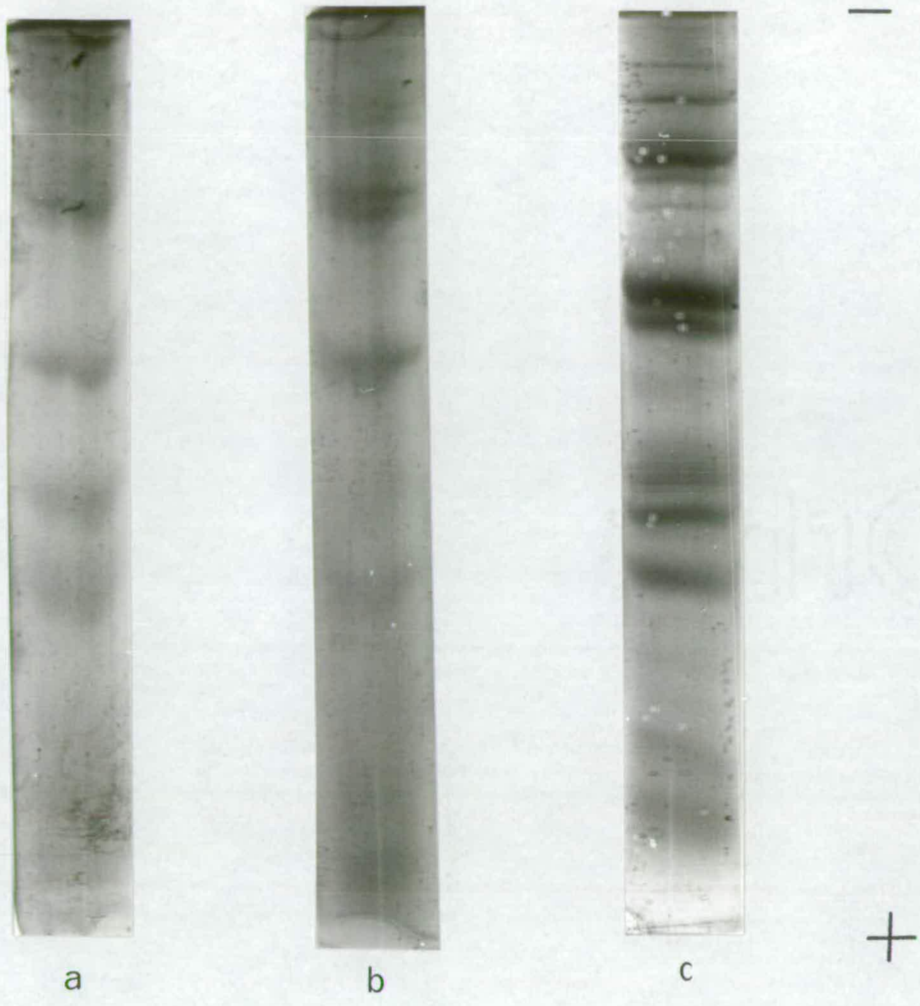
4) Species from the remaining sections tested all tend to show their own distinctive patterns which are quite different from those of the Vesicariae. C.aquatilis (Sect.Acutae) has a prominent series of well resolved high molecular weight components in addition to four of the low weight bands found in the previous species and in many of the other sections. C.laevigata (Sect. Elatae) and C.binervis (Sect.Spirostachyae) both tend to have very few visible bands, but it is probably significant that at least two of the major bands (from a total of four) match very closely, possibly supporting suggestions that these two species are rather closely related and should be placed in the same section (cf. David, 1974). Anatomical and cytological evidence also support this point of view (cf. Chs. 5 & 6). The other species examined showed differences in pattern but it is not possible to reach any meaningful conclusions on relations since only a few species from a wide range of sections have been investigated. C.davalliana (Sect.Dioicae) and C.muricata

Fig. 4.3 Photographs of banding patterns produced by *Carex* seed proteins on SDS polyacrylamide gels.

a) *C.vesicaria*

b) *C.saxatilis*

c) *Eriphorum angustifolium*



(Subgen.*Vignea* Sect.*Muehlenberginae*) show very little resemblance in their banding patterns. This is of some interest in view of the debate over the affinities of Sect.*Dioicae* and other members of Kukenthal's subgenus *Primocarex*. This section has been placed in subgenus *Vignea* by Kreczetowicz (1935) and also by Kern & Reichgelt (1954). There is no obvious similarity between *C.davalliana* and *C.muricata*, but it is possible that some of the other sections of Subgen.*Vignea* might be more closely related to the *Dioicae* since they are reported to hybridise with *C.dioica* at least, whereas no members of Sect.*Muehlenberginae* are known to hybridise with the *Dioicae*. *C.pilulifera* gave the best gels obtained from *Carex* material, with about 15 well resolved bands and were almost comparable in clarity and strength with those from *Eriophorum*, although the latter had more numerous bands.

The urea gel system was tested using an extract from *C.rostrata* and gave promising results with 10 clearly resolved, distinct bands which were generally much denser and sharper than those obtained on SDS gels. It would appear that for *Carex* material, the urea gel system is very effective and produces superior separation of seed proteins to the SDS gels. It is likely, however, that these gels will reveal more intricate small-scale variation leading to greater problems in interpretation. There was no time to test this system on any other *Carex* species.

4.5 Discussion and conclusions

The use of serological techniques in the present study has not been very productive and it has not been possible to produce an effective antiserum. Two possible explanations for this failure are that:-

1) The amount of protein present in the NaOH extracts was greatly overestimated. As mentioned earlier, it is possible that phenolic compounds and tannins may have interfered with the Lowry reagents, leading to overestimation of the protein content of the extracts used for antiserum production. The only method of avoiding this problem is to add a reducing agent (ascorbic acid or mercapto-ethanol) to prevent polphenol production and polyvinylpyrrolidine to remove tannins during the extraction procedure (cf. Harborne, 1973). It would, however, be necessary to remove these compounds before the extract was injected into an animal, and this could lead to complications in the procedure if sufficiently pure antigen solutions are to be obtained.

2) Carex seed proteins are not strongly antigenic. It is known that some proteins may not be strongly antigenic and it is possible that some of the major seed proteins in Carex do not induce a very powerful immune response in rabbits. This could be a result of the protein structure or of denaturation of the proteins by the extraction technique. Although some reaction was detectable with proteins dissolved in NaOH, it seems probable that extraction in such a highly reactive solventmay cause considerable damage. It was known that glutelins have not been widely used in serological investigations, but in Carex, these appear to be the only protein class available in sufficient quantities.

It is possible that antisera could be produced by modifying the techniques employed in the present study. If abundant material was available, concentration of a large volume of dilute extract might be attempted, but in many Carex species it is difficult to obtain the large seed quantities required. Even material intended as antigen for double diffusion tests only will still need to be concentrated to provide clear reactions. Another possibility is the use of extractants containing urea or detergents in the hope that these would provide a fairly concentrated solution of proteins which were still antigenically active after removal of the urea or detergent. It is not known whether intact Carex proteins can be obtained by these methods and at present, it would appear that the alternative technique of gel electrophoresis offers a much faster and more convenient method of examining seed proteins in Carex.

The results obtained from gel electrophoresis suggest that this technique may be extremely useful both for comparison of related species and for investigations at sectional or higher levels. In agreement with biosystematic evidence, C.vesicaria and C.saxatilis appear to be very similar. C.rostrata, however, appears to be rather distinct, also agreeing with anatomical and cytological evidence, while C.pseudocyperus shows some resemblance to C.vesicaria, suggesting a link between the Pseudocypereae and the Vesicariae. The members of other sections tested all appear to produce distinctive banding patterns on gels, indicating that gel electrophoresis may be a powerful method of obtaining information of relationships at sectional level. The excellent results obtained with Eriophorum angustifolium suggest that SDS gel systems may be particularly suitable in this genus, and it is possible that they could be successfully employed in studies of other genera in the Cyperaceae where generic and sectional delimination is very difficult, e.g. Scirpus (cf. Oteng-Yeboah, 1972).

The survey described in this chapter is very much a preliminary exploration of the applicability of protein studies and PAGE to problems in Carex and the Cyperaceae in general, and a number of important qualifications must be made concerning the results reported here. Considerable problems still remain in the interpretation of the banding pattern produced, particularly in deciding whether or not bands are homologous. These could be reduced somewhat by the use of slab gels in which a large number of samples are run in a single homogeneous gel slab (cf. Harborne, 1973). This allows correlations to be made between bands with greater confidence since differences brought about by variations between individual gels are removed. It is also possible to test homology to some extent by running a mixture of extracts and comparing these with the gels obtained from pure extracts. If bands are homologous, they should form a single band in a 'mixed' gel. Even if two bands appear to be homologous by the latter test, they may have different amino acid compositions but this could only be detected by peptide 'fingerprinting' or complete amino acid analysis and sequencing of purified proteins - a long and complex process which is at present impracticable for large numbers of species. However, even without the latter steps, apparent homology of banding should indicate some degree of resemblance between species since it is unlikely that two completely different proteins would have exactly the same mobilities, even on SDS gels. In the present survey, I have tried to use overall similarity of banding patterns rather than trying to determine exact homologies between bands because it seems likely that if the overall patterns shown by two species are very similar, it is unlikely that the individual bands making up the patterns are composed of totally different proteins. In a detailed study, however, it would be essential to investigate the homology of the protein bands more closely.

The range of material examined at present is insufficient to allow any detailed assessment of the reliability of protein methods in Carex. At present, no studies have been made on variation within species: it is possible that in C.rostrata at least, different clones may possess significantly different protein compositions. Plant species appear to vary widely in the degree of intraspecific seed protein variation which they demonstrate. From serological studies, Smith (1975) concluded that samples of Bromus hordeaceus from different continents, though from similar habitats, were indistinguishable in seed globulin constitution. In the same species, inter-habitat comparisons revealed differences in proteins. There is now abundant evidence that variation in protein content occurs in a number of species, e.g. in Phlox (Levin & Schaâl, 1972; Levin, 1975) and Chenopodium (Crawford, 1974) and variation in isoenzyme systems has been demonstrated in a wide range of organisms (cf. Hunziker, 1969). A review of the use of isoenzyme variation in zoological systematics is given by Avise (1974) and Lewontin (1974) and intraspecific isoenzyme variation as well as variation in non-enzymatic proteins has been examined in Betula by Payne & Fairbrothers (1973). In this case, pollen proteins were used, and it is possible that these are more variable than seed proteins. It is obvious that the possibility of widespread intraspecific variation cannot be ignored.

Although SDS gels seem preferable from a theoretical viewpoint, they did not give very good band resolution in a number of Carex species, with many rather faint, diffuse bands. The reasons for this are not known but it is possible that some protein degradation occurred because of the activity of proteases during extraction. This would lead to formation of polypeptide fragments of varying molecular weight which in turn, would tend to produce broad, diffuse bands. The fast moving, diffuse bands found on many of the gels may be the result of protein breakdown and may therefore be less significant than the better resolved, slower moving high molecular weight bands. The urea gels tested on C.rostrata extracts gave excellent results and might be preferable where detailed examination of a large number of sharply defined bands is required. For future studies of Carex seed proteins, three main recommendations can be made:

1) Extraction should be carried out in as short a time as possible. 30-60 mins. should probably be sufficient to obtain an adequate protein yield for gel electrophoresis. Extraction in NaOH may damage the proteins and if this solvent is used, a thorough check on the extent of denaturation is required.

2) The extractant should contain a reducing agent (ascorbic acid or mercaptoethanol) and polyvinylpyrrolidine to prevent the formation of complexes between the proteins and polyphenolic compounds and tannins.

3) The urea gel system of Panyim and Chalkey (1969) probably gives maximum resolution of large numbers of proteins for detailed study, but the SDS gel system provides complementary information and is probably of considerable value at sectional or higher level. Serological systems appear to give poor results with Carex seed proteins.

A wide range of PAGE techniques is now available, using a variety of buffer systems of differing pH concentration. It should therefore be possible in future investigations to choose a system which is especially suitable for the problem at hand, depending on the taxonomic level of the investigation. Very few studies appear to have been considered closely with the results obtained from the use of several gel techniques on the same material. Payne and Fairbrothers (1973) used a number of methods and concluded that each protein/gel system used showed different amounts of intraspecific variability. Schechter & De Wet (1975) working on cultivars in Sorghum came to a similar conclusion. It should therefore be possible to provide a range of gel systems which can be used to investigate problems at different taxonomic levels within the group being studied. SDS gels form one end of a spectrum of techniques which reveal protein variation in greater and greater detail, with detailed studies of isoenzyme distribution as the other end of the spectrum. Provided that their limitations are kept in mind, PAGE systems offer a flexible investigation system which can be adjusted to deal with taxonomic problems at a variety of levels in the taxonomic hierarchy.

Note. After the writing of this chapter was completed, an important collection of papers on plant proteins, edited by Harborne and Van Sumere (1975), became available. This contains a number of papers which are relevant to the present discussion. In particular, the papers by Daussant on immunological techniques, and those of Stegemann and Preaux & Lontie on protein components of potatoes and barley provide useful summaries of protein separation and identification methods and their application in practice. Stegemann's studies are of particular interest as he used a series of different acrylamide gel techiques to separate potato storage proteins. It is noteworthy that when an SDS gel system was used, there were very few differences visible between different potato varieties, but gel systems which separated proteins primarily on a charge basis showed wide differences.

This would seem to suggest that a large part of the variation seen in many gel systems may be a reflection of small scale variation in amino acid composition and charge distribution in what are essentially single proteins. Stegemann also gives examples of 2-dimensional gel separations where the proteins were first sorted by charge differences (by isoelectric focussing) and then separated by molecular weight in a second dimension. These showed that some proteins which were separated by the first treatment were composed of three subunits of differing molecular weights, but the subunits of each 'charge' group were identical in molecular weight. This strongly suggests that the different 'charge' groups were in fact minor variants of the same protein. Stegemann's findings would seem to confirm that SDS gel systems are less sensitive to minor variation in protein composition, and are probably more useful in comparisons of widely separated organisms, as the differences which they show probably reflect more substantial evolutionary changes in the protein complement.

The paper by Van Sumere <u>et al.</u> is also of considerable interest as they give a detailed discussion on the interactions between phenolic compounds and proteins during extraction from plant materials and also recommend methods of avoiding the problems which arise.

5.1 Introduction

This chapter summarises the survey of leaf and culm anatomy carried out on British and European species of the Vesicariae complex and a number of other sections. Numerous studies on various aspects of Carex anatomy, covering a wide range of species, have been published. These are listed by Metcalfe (1971) in his extensive bibliography. No attempt is made here to give a general review of anatomical studies throughout the genus because the enormous volume of published material makes such an undertaking impracticable in the present study. There are, however, several important surveys which have included members of the Vesicariae complex or members of sections examined in the present survey. These include Mazel (1891), Crawford (1910), Akiyama (1935, 1942), Le Cohu (1967) and Metcalfe (1971). The systematic value of anatomical characters at generic and higher levels in the Cyperaceae is discussed by Metcalfe (1969, 1971) and Oteng-Yeboah (1972).

5.2 Materials and methods

As far as possible, fresh material collected directly from the field or cultivated in the greenhouse was used. In many cases this was not available and herbarium material was used. This was treated by boiling for a few minutes in tap water with a little wetting agent (Teepol) added and could then be sectioned in the same way as fresh material. Sections were hand cut from the middle part of the uppermost leaf of the flowering stem but where no flowering material was available, a well-developed vegetative leaf was used. The sections were left unstained, because most of the details of the schlerenchyma and vascular tissues were easily visible without staining, and were mounted directly in Gum chloral. Drawings were made with a camera lucida and photographs were taken on a Vickers 'Patholux' microscope on Ilford Pan F film developed in Acutol. At least two or three specimens of each species were examined, and in the Vesicariae a larger number of specimens of each species (ca. 10 or more) were investigated to estimate the intraspecific variation range which exists. Culm anatomy was surveyed mainly within the Vesicariae complex, although a few other species were included. Sections were cut at the mid-point of the culm and treated in the same way as the leaf sections.

The epidermal structure of both adaxial and abaxial leaf surfaces was examined by means of cellulose acetate impressions. These were made by pressing a small square of cellulose acetate sheet (of the type used for overhead projection sheets) against a leaf surface which had been moistened with a drop of acetone. It was found that dissolving a small amount of scrap acetate sheet in the acetone prevented the solvent from drying out too quickly after it had been applied to the leaf surface. These impressions did not reveal so much detail as epidermal strips (which were also examined in a few cases), but they were much easier to use and showed cell shape, presence or absence of papillae and distribution of the stomata. Since this procedure could be carried out very easily and quickly, leaf impressions were used extensively, even for plants collected in population samples.

Scanning electron microscopy (SEM) was used to investigate surfaces in more detail to check the results obtained from the cellulose acetate impressions. Dried herbarium material was used, mounted on standard SEM specimen stubs with 'Durofix' glue and coated with 200-400 Å of gold or gold/palladium. These were observed in a Cambridge 'Stereo scan' microscope at 20 kV. Photographs were taken on Kodak Tri-X or Ilford HP4 film at various magnifications.

5.3 General features of Carex anatomy

General features of leaf, culm and rhizome anatomy in Carex have been described by Crawford (1910), Le Cohu (1967) and Metcalfe (1971) and I do not intend to give a detailed treatment here. Some explanation is, however, necessary, and the following account gives a brief description of the general structure of the leaf and culm. Most of the leaf structures are illustrated in Fig.5.1. The nomenclature follows Metcalfe (1971).

In section, the form of the leaf varies considerably from more or less crescent-shaped to variously flanged or inversely W-shaped types. There is a median vascular bundle (usually the largest in the leaf) and the two halves of the lamina on either side contain varying numbers of secondary vascular bundles which are almost invariably arranged in a single row nearer the abaxial surface of the leaf. Around the bundles of all the species examined, there was an inner fibrous bundle sheath and an outer sheath of large, more or less translucent cells. Between the vascular bundles and the adaxial and abaxial surfaces, there are normally two girders of schlerenchymatous tissue, forming an adaxial girder and an abaxial girder. These are very variable in shape as seen in cross section, although the shape is usually relatively constant within the species. In many cases the adaxial girder is incomplete and consists of a number of separate strands of schlerenchyma embedded in a column of outer bundle sheath cells. In certain species the adaxial girder is reduced to a small 'cap' on the bundle and a single triangular strand in contact with the adaxial epidermis, while in a few species, some of the smaller bundles may completely lack schlerenchyma tissues apart from the inner bundle sheath. The abaxial girder is normally much shorter and wider and less variable than the adaxial. Between the vascular bundles, prominent air spaces of varying size and shape are present, but they are normally

Fig. 5.1 Leaf structure in C.vesicaria. (x225)

a) & b) secondary vascular bundle

c) & d) median vascular bundle

ad. adaxial schlerenchyma girder

ab. abaxial schlerenchyma girder

as. air space

os. outer bundle sheath

is. inner bundle sheath

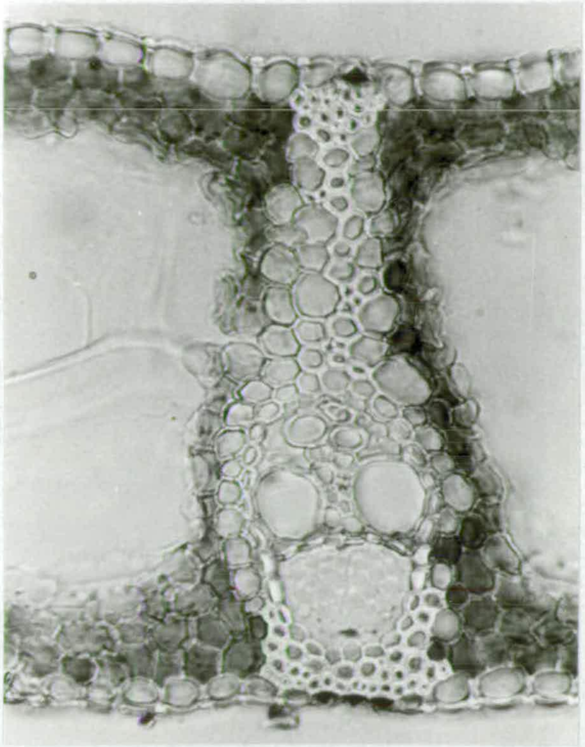
x. xylem

p. phloem

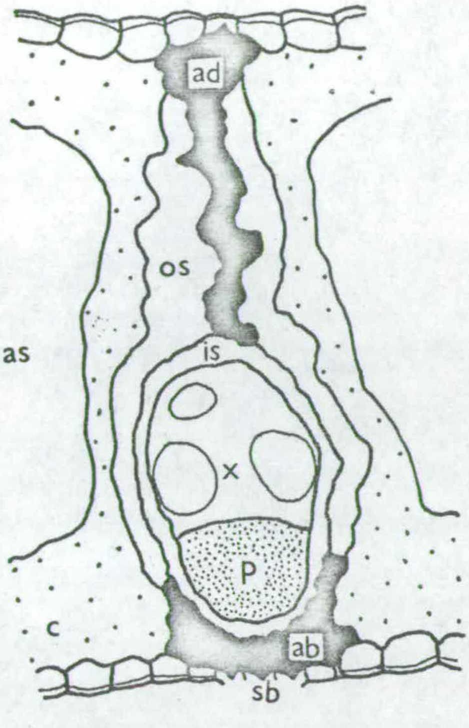
sb. silica body

c. chlorenchyma

b. bulliform cells



a



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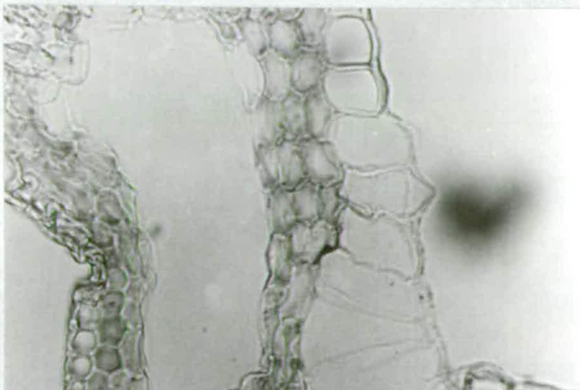

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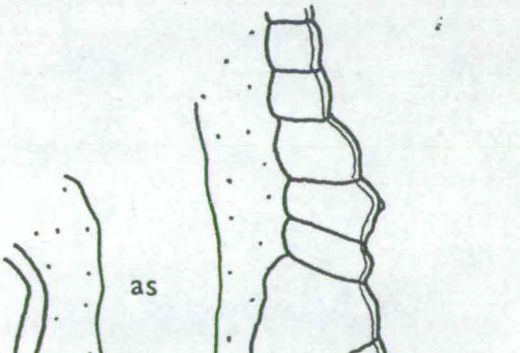


d

rectangular and occupy a large proportion of the interior of the leaf. The chlorenchyma forms a layer of tissue (1) - 4 or 5 cells thick, lining the interior of the air spaces. There is usually a single layer of distinct, enlarged bulliform cells in the epidermis above the median vascular bundle, but some species also have a hypodermal layer of large translucent cells below the bulliform cells. Stomata were predominantly abaxial and were paracytic in all the species examined. Certain species have papillate epidermal cells and scattered low papillae may be found near the leaf edges in many species which are otherwise totally non-papillate. Conical silica bodies like those described by Mehra & Sharma (1965) were found in almost all the species examined. These occurred only in epidermal cells adjacent to schlerenchyma fibres. The outer cell wall in the silica cells was frequently very thin and in dried specimens tended to collapse, leaving a row of projecting conical bodies with folds of outer cell wall between each silica body as can be clearly seen in Fig.5.2. These rows of silica bodies were often detectable in cellulose acetate impressions, but in species where the outer cell wall is thicker (e.g. C.rostrata), silica bodies were not visible using this technique.

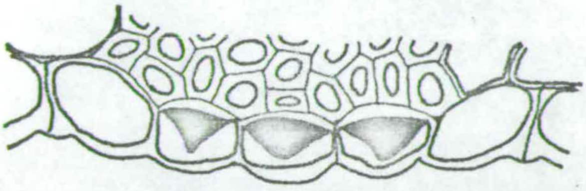
The outline of the culm sections varied from acutely triangular to almost circular or irregular. In culms there is normally a more or less regular peripheral ring of large vascular bundles which alternate with the peripheral air spaces, the latter varying from regularly rectangular to much more irregular shapes. In addition to the outer vascular bundles, there are often numerous scattered bundles which may be larger or smaller than those in the outer ring. The outer vascular bundles are usually connected to the epidermis by schlerenchyma girders which vary considerably in shape and size.

Fig. 5.2 Silica bodies in C.vesicaria and C.rostrata.

a) T/S abaxial epidermal cells adjacent to a schlerenchyma girder in C.vesicaria. Note the thin outer cell walls which collapse easily. (x 650)

b) The same structure in C.rostrata. The outer cell walls are much thicker and do not collapse easily. (x 650)

c) Scanning electron micrograph of cells containing silica bodies in the abaxial epidermis of C.vesicaria. The outer cell wall has collapsed and forms folds of material between the projecting silica bodies. (magnification approx. x 2000).



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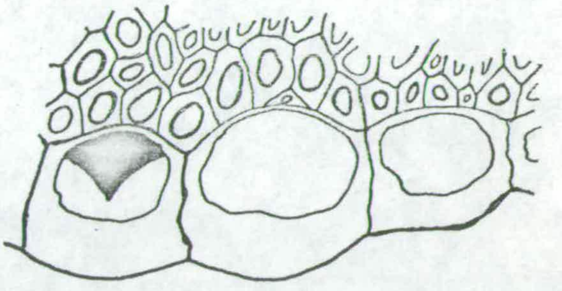

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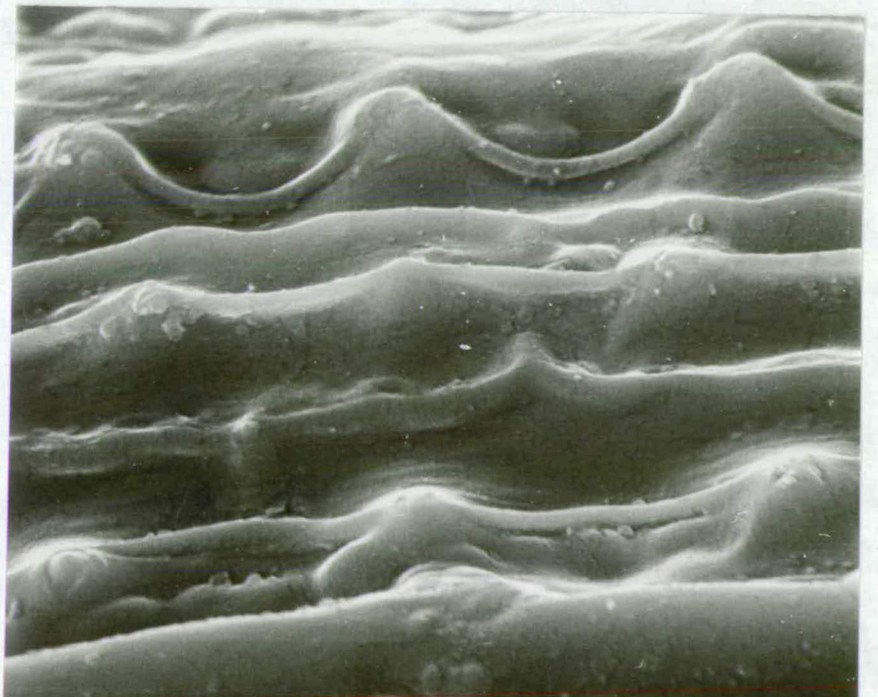

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The central bundles sometimes have small schlerenchyma caps at the xylem and phloem poles but these are frequently poorly developed or completely absent. The outer bundle sheath is obscure but the inner sheath was always present in the material examined in this survey. The chlorenchyma tissue varies in thickness but usually extends inwards to the inner edge of the peripheral air spaces. There is, however, no sharp boundary between the chlorenchyma and the inner, translucent cells. Most species have a large central air space.

5.4 Anatomy of species in Sect.Vesicariae

The four British species of this section were investigated in some detail for both culm and leaf anatomy. In the following descriptions, the terms used follow Metcalfe (1971) although in some cases, they are a little cumbersome.

5.4.1 C.rostrata (Figs.5.3; 5.6; 5.9; 5.12; 5.13; 8.2; 8.10)

T/S lamina V-shaped to slightly flanged; keel rounded to acute, prominent, lamina thickest at middle of each half, tapering to midrib and margins; median axial groove small but distinct, bulliform cells in a single layer, large and prominent. Adaxial epidermal cells slightly larger than abaxial; strongly papillose with 1 papilla/cell while the abaxial epidermis is non-papillate; cells over schlerenchyma girders smaller than the rest and containing 1-3(-4) conical silica bodies in a single row, with thick outer walls. Stomata adaxial, surrounded by a circle of papillae which enclose the guard cells in a cup-shaped depression (Figs. 5.12a; 8.10). Median vascular bundle assymetrically placed, with a descending crescentiform abaxial schlerenchyma girder and a wing which descends into the keel (Fig.5.9a) or with a separate triangular girder running along the keel (Fig.5.9b); an adaxial cap is present. Lateral bundles ca.10 (more numerous in wide-leaved forms) with irregularly sub-T shaped adaxial girders and descending crescentiform to broadly Y-shaped abaxial girders. In a few cases, the adaxial girders were found to be broken up into a series of separate strands (fig.5.6a) but in most of the specimens examined they were complete. The outer bundle sheaths extend adaxially almost to the epidermis and ± completely enclose the schlerenchyma girder. The outer cell walls of the abaxial epidermis are thicker than in C.vesicaria and as a result, the silica bodies do not normally show up in cellulose acetate impressions. The air cavities are ± square or shortly rectangular, large.

T/S culm ± triangular, with convex sides and obtuse angles; central cavity large and irregular. Peripheral air cavities ca. 20-25, ± square or irregular. Chlorenchyma extending inwards to between the vascular bundles and air spaces. Vascular bundles ca.30, in an outer regular ring with scattered larger bundles inside, embedded in translucent parenchyma. Schlerenchyma girders broadly securiform for outer bundles, occasionally small crescentiform caps on the inner bundles.

The above description refers to typical more or less narrow-leaved European material, which generally appears to be fairly uniform. Some specimens of var.utriculata from North America and in the wide-leaved European forms were also examined and found to differ from the type variety in several respects. The wide-leaved European forms were similar to the type in their epidermal characters, although the papillae on the adaxial epidermis tended to be more widely spaced. The leaves were much wider and thicker (Fig. 5.3d) and tended to be of the same thickness along most of the length of the section in contrast to the narrow leaved forms which were usually markedly thickened in the middle of each half lamina. The number of vascular bundles appeared to be the same or slightly greater (ca. 10-11 in each half of the lamina) but the schlerenchyma girders on the adaxial side were very narrow and elongated and almost always broken into two or more strands. The abaxial girders tended to be wider at their contact with the epidermis. Below the bulliform cells, there was an irregular layer of large, translucent cells, a character not noted in any of the narrow leaved specimens. The culms in both of the specimens studied were much thicker than in the typical form and were more irregular in shape, with larger irregular air spaces - both central and peripheral - especially in Praeger's specimens from W.Ireland (this was the plant which was initially recorded as C.laevirostris). (Figs. 5.13b,c). In addition, the large inner vascular bundles were more numerous and better developed and the schlerenchyma girders supporting the outer bundles were shortly T-shaped to securiform.

The American material of var.utriculata showed more marked differences in anatomy of both leaves and culms. The epidermal structure in var.utriculata is more similar to C.vesicaria than to the remainder of C.rostrata as papillae were completely absent and the stomata were abaxial and not enclosed in a ring of papillae. The adaxial epidermal cells also tended to be noticeably larger than the abaxial cells. The leaves were very much wider and thicker than in the type variety but the material was very variable and at least one of the specimens examined (K 38 - see Appendix 1) was similar in overall size to the European forms. The number of vascular bundles tended to be higher, varying from 11 to 16, and, as in the broad-leaved British forms, the adaxial girders were very slender and almost invariable broken into a series of separate strands (fig.5.6c,d). Below the bulliform cells, a layer of large translucent cells was again present, but in this case, they were much more regularly arranged and more strongly developed than in the European types (Fig.5.9c,d - note that these are not extreme examples of the hypodermal development). The culm shape is very variable, ranging from approximately triangular with irregular, ridged sides to more complex shapes. The air spaces were less regular and the large inner bundles more frequent than in the type variety. The schlerenchyma girders attached to the outer bundles were sucuriform to broadly baculiform or Y-shaped. Smaller specimens from north and eastern Canada similar in size and appearance to the European forms were found to have a papillate adaxial epidermis and adaxial stomata surrounded by a ring of papillae and thus agree with the type variety in epidermal anatomy, but the general leaf and culm anatomy has not been examined in these forms. It would appear, however, that two distinct anatomical variants of C.rostrata occur in North America and that these are to a certain extent correlated with morphological characters. These findings are discussed in more detail in chapters 8 and 9. The papillate epidermal structure found in European material of C.rostrata appears to be a very constant character since it has been observed in more than 150 specimens of this taxon from different parts of Europe and Western Asia. Eastern Asiatic specimens tended to have the var.utriculata structure. These were examined by use of cellulose acetate impressions.

The characteristic band of prickle cells above the insertion of the ligule, described by Le Cohu (1970), was found to be present in British material (3 specimens examined) and was also found in var. utriculata specimens from North America. This character might also be of value in separating other species pairs.

5.4.2 C.vesicaria (Figs.5.1; 5.3; 5.14; 8.2; 8.10).

T/S lamina ± flanged or inverse W-shaped to flat; keel prominent, rounded, lamina of even thickness or slightly thicker at the middle; median axial groove not well developed, bulliform cells in a single layer, large and prominent. Adaxial epidermis smooth, cells larger than in abaxial but not markedly so, abaxial epidermis also smooth; cells over girders smaller than the rest, containing 1-4 conical silica bodies in a single row, outer cell wall thin. Stomata abaxial, although a few scattered adaxial stomata are usually present, not surrounded by papillae (Figs.8.8; 8.11). Median vascular bundle assymetrically placed, with a descending crescentiform abaxial girder with a wing extending into the keel (Fig. 5.1) or with a separate triangular keel strand; an adaxial cap is present. Lateral bundles ca. 11-12, with irregular sub-T-shaped or narrowly triangular adaxial girders, sometimes broken into separate strands, and broadly crescentiform or pulviniform abaxial girders. Outer bundle sheaths extend adaxially to the epidermis and completely enclose the girder. Silica bodies easily visible in cellulose acetate impressions because of the thin outer cell walls. Air cavities rectangular, large.

T/S culm triangular, with acute angles, sides concave; central cavity large and irregular. Peripheral air cavities ca.25, variable in size and shape, chlorenchyma extending to inner side of cavities. Vascular bundles 28-33, mainly in an outer regular ring and a few large bundles in an irregular inner ring. Girders of outer bundles varying from tall securiform to broadly securiform, with triangular strands in the angles of the culm.

North American material of this species has not been investigated. There was some variation in the shape of the adaxial girders in the leaf which ranged from T-shaped to narrowly securiform, but this species generally appeared to be more uniform than C.rostrata. It is likely, however, that a wider survey would reveal a much greater variation range. Cellulose acetate impressions were made for over 50 specimens from different parts of Britain and Europe. Almost all of these had smooth, non-papillate epidermal structures and the few which did possess papillae were intermediate between this species and the preceding one in morphology and were probably hybrids.

5.4.3 C.saxatilis (Figs.5.3; 5.6; 5.9; 5.12; 5.14; 5.16)

T/S lamina variable in outline but usually flat or somewhat thickened at the middle of each half of the lamina to give a V-shaped or slightly flanged type, often rather abruptly rounded at the tips; keel rounded, not prominent; median axial groove variable, sometimes well-developed, bulliform cells in a single layer, usually large and prominent but in some cases not much larger than the surrounding epidermal cells. Adaxial epidermis smooth, non-papillate, cells usually markedly larger than those of the abaxial epidermis which is also smooth; cells over schlerenchyma smaller in abaxial epidermis, containing 1-4 conical silica bodies, but of $\pm$ constant size and rarely with silica bodies in the adaxial epidermis. Stomata abaxial, not surrounded by papillae (Figs. 5.2; 5.12e). Median vascular bundle more or less central, with a descending crescentiform abaxial girder (assymetric winged girder if bundle assymetrically placed Fig. 5.9i,j); an adaxial cap is present. Lateral bundles (5-) 6 - 7, with triangular to shortly baculiform adaxial strands and an adaxial cap on the bundles, occasionally with additional strands between the two; abaxial girder shortly securiform to pulviniform. Outer bundle sheath extending to adaxial epidermis or stopping at the adaxial schlerenchyma. Air cavities fairly regular, square to rectangular, slightly smaller relative to the leaf thickness than in the preceding species.

T/S culm irregularly triangular, with rounded, obtuse angles; central cavity ± oval, smaller than in the previous species. Peripheral air cavities ca.12-18, irregular, frequently coalescing to form larger cavities. Chlorenchyma extending to inner edge of air cavities. Vascular bundles 22-24, in an irregular outer ring, with a few larger bundles scattered in the inner parenchyma. Girders broadly securiform to turbiniform, with ± triangular strands in corners not occuped by bundles, crescentiform caps on inner bundles.

The European material of this species is quite variable, with the outline of the leaf T/S varying from more or less channelled to almost flanged. The adaxial schlerenchyma was developed to a varying extent, with very small separate strands frequent in some plants. The abaxial girders were entirely absent in a few of the bundles. A rather tall, peduncled form from Ben More (Perthshire) was compared with the normal variety from the same locality and although the leaves were flatter and the air spaces in the culm more irregular, the differences observed were not very great and probably well within the normal variation range for the species. A specimen from a collection made in Labrador was also examined and found to show some interesting differences from European material. Only the leaf anatomy was examined in this case. The leaf was narrower, but of the same thickness while the adaxial epidermal cells were not so obviously larger than the abaxial cells. The adaxial schlerenchyma strands were narrowly triangular to sub-T-shaped and almost formed a complete girder, together with the Y-shaped cap on most of the bundles. A Y-shaped cap was also present on the median bundle. A hypodermal layer of translucent parenchyma cells was also present below the bulliform cells. (Figs. 5.3i; 5.6j; 5.9h).

5.4.4 C. grahamii (Figs. 5.3; 5.6; 5.9; 5.14; 5.16)

T/S lamina Channelled or slightly flanged; keel rounded, not prominent, lamina $\pm$ flat or slightly thickened at the middle of each half; median axial groove variable but usually not well developed, bulliform cells in a single layer, large and prominent, often with a thin layer of translucent cells below them. Adaxial epidermis smooth, non-papillose, cells larger than those of the abaxial epidermis which is also smooth; cells over schlerenchyma smaller than the rest, containing 1-4 conical silica bodies in a single row (usually mainly in the abaxial epidermis). Stomata abaxial, not surrounded by papillae. Median vascular bundle more or less central with a descending crescentiform abaxial girder (winged crescentiform or with a separate triangular strand in the keel if the bundle is assymetrically placed); an adaxial cap is present. Lateral bundles ca. 8, with triangular to sub-T-shaped adaxial strands, a crescentiform or shortly Y-shaped adaxial cap and 1-3 separate strands (the development of the adaxial girder is very variable); abaxial girder narrowly securiform or inversely securiform. Outer bundle sheath extending to adaxial epidermis. Air cavities regular, square or rectangular.

<u>T/S culm</u> $\pm$ triangular but with very rounded corners, sometimes almost subterete; central cavity large, oval or $\pm$ triangular, larger than in <u>C.saxatilis</u>. Peripheral air cavities ca.20-24 rounded, oval or irregular but usually forming a fairly regular ring. Chlorenchyma not quite extending to inner face of air cavities. Vascular bundles ca.25-27, in a regular outer ring with a few larger bundles displaced inwards but not forming a distinct inner ring. Girders broadly securiform to turbiniform, inner bundles with small crescentiform caps; $\pm$ triangular or irregular strands in the corners of the culm.

The above description refers to material from the type locality at Glen Clova. Material from the population on Meall nan Tarmachan was also examined and found to show some differences in details of the leaf and culm anatomy but these were not very large. The Tarmachan material tended to have a smaller difference in size between the adaxial and abaxial epidermal cells and the bulliform cells were usually smaller and did not occupy such a large part of the median area of the leaf. As in the Clova material, the size and shape of the adaxial girders was variable. The culm was more definitely triangular in outline, often with at least one acute angled corner, and was thinner, with fewer vascular bundles and air spaces (21-25 and 18-20 respectively). (Figs. 5.3j; 5.6g; 5.9f; 5.14 c,e).

From the above, it can be seen that the British members of Sect.Vesicariae are quite diverse in their anatomy, particularly in epidermal and shape characters. Observations made in the present study generally agree well with those of previous investigators although there are some differences. Le Cohu (1970) noted papillae on both leaf surfaces in C.rostrata. In the present study, this was only found in a few individuals which were thought to be hybrids (cf. Ch.8) and it is possible that some of the specimens studied by Le Cohu were in some way aberrant. Otherwise, the agreement between observations is good and any minor differences are probably well within the natural variation range.

It is difficult to find a set of characters which consistently separate members of Sect.Vesicariae from the remainder of the sections investigated, but the species of this section tend to have a few characters which give the leaf anatomy a distinctive appearance in many cases. The following are common to most of the Vesicariae:

1) The adaxial schlerenchyma girders are usually rather tall and slender, (sub T-shaped) or if the girder is reduced to separate strands, these are not very large and the columns separating the air

spaces consist of the extended outer bundle sheath and a very thin layer of chlorenchyma, together forming rather tall slender columns of a shape which is rare in any other sections.

2) The outer bundle sheath almost invariably extends to the adaxial epidermis, enclosing the adaxial girder.

3) The bulliform cells form a small group above the median vascular bundle and do not extend much over the lamina. This, however is by far the commonest state found in Carex and is by no means confined to the Vesicariae.

4) With the exception of C.rostrata, all the members of the Vesicariae so far investigated have non-papillate epidermal structures with abaxial stomata. This includes C.laevirostris and C.rotundata (Akiyama, 1935; 1942). This is again a character which is common in the genus as a whole.

The anatomy of C.grahamii is of considerable interest in view of the speculations surrounding the origin of this species (cf. Ch.2). In general, there is comparatively little direct evidence to support any one of the suggestions, but the anatomy is at least compatible with an origin from a hypothetical C.saxatilis x vesicaria hybrid. In both leaf and culm anatomy, the Meall nan Tarmachan material is almost directly intermediate between the two species, although the leaf shape is closer to C.saxatilis. The differences and similarities between C.grahamii and the possible parental species are summarised in Table 5.1. Differences in development of schlerenchyma girder shapes and the bulliform cells have not been very helpful in this case since there is wide variation in all of the taxa considered and the C.grahamii material falls within the variation range of both C.vesicaria and C.saxatilis. Only C.rostrata shows significant differences in having

Table 5.1 Anatomical characters in British species of Sect. <u>Vesicariae</u>

Character	1	2	3	4	5	6	7	8
C. rostrata	-	+	-	±	-	9-11	30-35	20-25
var. *utriculata*	+	-	+	+	-	11-16	26-46	19-30
C. vesicaria	+	-	++	-	-	11-12	28-33	20-25
C. saxatilis	+	-	+	±	+	5- 7	22-24	12-18
C. grahamii (Clova)	+	-	+	-	+	7- 8	25-27	20-24
C. grahamii (Tarmachan)	+	-	+	-	±	7- 8	21-25	18-20

1. Stomata abaxial
2. Papillae present
3. Silica bodies obvious (in cellulose acetate impressions)
4. Hypodermis present (below bulliform cells)
5. Adaxial epidermal cells larger than abaxial.
6. Number of vascular bundles in each half of the lamina.
7. Number of vascular bundles in the culm.
8. Number of air spaces in the culm.

C. rostrata var. *utriculata* is included for comparison with the type variety.

girders which are normally complete and not broken into strands. There is no obvious trace of this characteristic in the C.grahamii material. The Glen Clova clone differs from the Tarmachan material in having relatively larger adaxial epidermal cells, a character which places it nearer to C.saxatilis and the more rounded shape of the culm also shows some resemblance to the latter species, but the more numerous vascular bundles and comparatively larger central air space are not typical of C.saxatilis.

The possibility that C.rostrata could be one of the parental species of C.grahamii would seem to be ruled out by the total absence of papillae on both of the epidermal surfaces of C.grahamii in both of the clones sampled. It could be argued that the presence of papillae and adaxial stomata are controlled by recessive genes which would not be expressed in a C.saxatilis x C.rostrata hybrid, but the presence of papillae in C.vesicaria x C.rostrata hybrids and possible backcrosses with C.vesicaria (cf. Ch.8) would seem to indicate that this is unlikely.

5.5 Anatomy of other sections of Carex

The leaf anatomy of the remaining Carex material which was examined is summarised in Figs. 5.4, 5.5, 5.7, 5.8, 5.10, 5.11, 5.12 and 5.16. Culm anatomy was also investigated in a few species (Fig. 5.15). In total,18 species in 14 sections were examined, with most emphasis on the sections most closely related to the Vesicariae. Full descriptions have not been given because these would occupy considerable space and it is only intended to describe the more significant variations from the pattern described in the Vesicariae. Species placed in the same section on morphological grounds have as far as possible been placed close together in the illustrations to facilitate comparisons within and between sections. In the following account, the sections have been split into two groups:-

1) those belonging to the Vesicariae complex - Sects.Hirtae, Pseudocypereae, Paludosae and Lupulinae.

2) other sections, including members of subgenus Vignea.

5.5.1 Sections of the Vesicariae complex

1) Sect.Hirtae.

Two species were examined, C.hirta and C.lasiocarpa. (Figs.5.4; 5.7; 5.10; 5.12; 5.15 and 5.17). It is obvious that these two species differ considerably in their leaf anatomy as can be seen from Figs.5.4; 5.7; 5.10 and 5.15. In the overall shape of the leaf section, C.lasiocarpa is thickly crescentiform - a shape which is comparatively rare among British Carex species - while C.hirta has an inversely W-shaped to flanged V-shaped section. C.lasiocarpa had an unusual type of adaxial epidermis in which most of the cells were enlarged and there were no clearly defined bulliform cells. This was seen in an even more extreme form in C.extensa (section 5.5.2). There were occasional large prickle cells on the adaxial epidermis in some specimens. In contrast, C.hirta had a normal epidermal type with enlarged bulliform cells and the rest of the epidermis composed of smaller cells. The only unusual feature was the presence of papillae on the adaxial epidermis and unicellular hairs of varying length over much of the leaf surface. C.lasiocarpa had a hypodermal layer of large, translucent cells over the median vascular bundle but these were absent in C.hirta. Stomata in both species were abaxial. C.lasiocarpa resembled members of the Vesicariae in having rather tall, narrow abaxial girders, but these were usually broken into several separate strands whereas C.hirta had much broader girders which were normally divided into a bundle cap and a triangular to bulbiform strand underlying the adaxial epidermis.

The culm anatomy of the two species also differs. C.lasiocarpa had a much more rounded culm with the majority of the vascular bundles in a somewhat irregular outer ring while C.hirta had more numerous inner bundles and less elongated bundles and girders, which were usually turbiniform to pulviniform as opposed to the turbiniform or baculiform girders of the former species.

C.hirta had a leaf structure which was not very dissimilar from member of Sect.Vesicariae, but differed in its generally wide, short schlerenchyma girders, rather smaller air spaces and the presence of unicellular hairs on most parts of the plant. As indicated above, C.lasiocarpa was more distinctive and differed both from the Vesicariae and from C.hirta.

2) Sect.Pseudocypereae.

Only one species was examined - C.pseudocyperus. (Figs.5.4; 5.7; 5.10; 5.15; 5.16). The most obvious differences from the Vesicariae were in the thicker, shorter schlerenchyma girders, smaller air spaces and highly enlarged cells of the adaxial epidermis. The bulliform cells were more enlarged than in most species of the Vesicariae. Apart from these characters, however, the leaf anatomy of C.pseudocyperus was very similar to that of the latter section, and in particular C.vesicaria. The culm anatomy in C.pseudocyperus was more distinctive as there was no central air space and the numerous outer vascular bundles formed a very regular ring, with prominent, well-developed schlerenchyma girders which were ususlly securiform to turbiniform in shape. A large number of inner bundles were also present, forming an irregular ring, and most of these had prominent schlerenchyma caps.

3) Sect. Paludosae.

Only one species was examined - C. acutiformis. (Figs.5.4; 5.7; 5.10; 5.15; 5.16). This species differed from the Vesicariae in a number of respects. A prominent hypodermis was present below the bulliform cells, consisting of one or two layers of large, translucent cells. This species had the most obvious and regular hypodermal layer of all the species included in the present survey. The abaxial epidermis was strongly papillate and the guard cells of the stomata surrounded by a ring of papillae. The adaxial schlerenchyma girders were usually divided into a cap on the vascular bundle and a broadly triangular or securiform adaxial strand. The bundles were relatively larger and closer to the centre of the lamina than in members of the Vesicariae and the air spaces tended to be smaller. As in C. pseudocyperus, the culm contained numerous vascular bundles in an outer ring, but these were slightly less regular and the subtending turbiniform to securiform girders often extended around the bundles to join up with the inner cap to form an almost complete sheath around the bundles. A central air space was present, and the inner bundles tended to have an almost complete outer sheath of schlerenchyma.

4) Sect. Lupulinae.

Two species were examined - C. lupulina and C. grayii (Figs.5.4; 5.7; 5.10). These were found to be generally very similar to members of the Vesicariae. C. lupulina in particular had the tall, T-shaped adaxial girders typical of most members of that section, but C. grayii has wider girders which were usually divided into a cap on the vascular bundle and a more or less triangular strand. The bulliform cells were slightly less enlarged and the adaxial girder of the median vascular bundle was rather more massive in both species, but there were few distinctive characters separating these two species from the Vesicariae. Culm anatomy was not investigated.

5.5.2 Other sections.

The sections sampled belonging to subgenus Carex were Acutae, Montanae, Limosae, Paniceae, Spirostachyae, Flavae, Glaucae and Elatae, and in subgenus Vignea the sections were Ammoglochin and Paniculatae. Culm anatomy was examined only in Sects.Acutae and Montanae.

1) Sect. Acutae.

One species was examined - C.aquatilis. Material from typical forms of this species, originally collected in N.E. Scotland were compared with a much larger form occurring in S.W. Scotland. It was found that considerable differences existed between these plants, but they could be distinguished clearly from members of the Vesicariae.

The northern forms were examined both from cultivated material and herbarium specimens and it was found that the leaves varied from a narrow V-shape to a much wider, flatter leaf type (fig. 5.5h,i). There were also differences in the size and shapes of the adaxial girders and leaf thickness (Fig.5.8g,h). The large form from S.W. Scotland approached C.acuta in many of its morphological characters, but in leaf anatomy it resembled the larger of the northern forms, although the schlerenchyma girders were more strongly developed (Fig.5.8d) and there were minor differences in the midrib area (Fig.5.11d,e). The culms differed greatly in size and in the shape of the outer air spaces and the number of vascular bundles but were otherwise similar (fig.5.15g,h). In spite of the variation noted, there were strong similarities between the different forms, including the presence of a strongly papillate adaxial epidermis (in contrast to the abaxial papillae reported for C.acuta by Metcalfe (1971)) and the general appearance of the vascular bundles and related girders was fairly constant. The most obvious differences from Sect. Vesicariae were the usually thinner leaf with shorter or more interrupted adaxial girders and the more numerous vascular bundles in the inner parts of the culm. The leaf texture tended to be distinctive, being rather soft and flexible in the wider leaved forms, making section cutting without embedding more difficult.

2) Sect. Montanae.

Two species were examined - C.caryophyllea and C.pilulifera (figs.5.5b,d; 5.7i,j; 5.10i,j; 5.15f). The leaf anatomy of these two species was quite similar although minor differences occurred in the shape and size of the schlerenchyma girders. The main differences from the Vesicariae were the rather short, wide pulviniform to securiform adaxial girders and the restriction of the outer bundle sheath to the area immediately surrounding the bundle. The leaves tended to be narrow and thin, with relatively small air spaces, and the bulliform cells in C.caryophyllea were very small and not well differentiated from the rest of the epidermis. The culm in the latter species had no central air space and the vascular bundles were arranged, with few exceptions in a more or less regular outer ring. The outer air spaces were small and irregular.

3) Sect. Limosae.

One species was examined - C.paupercula (Figs.5.5g; 5.7h; 5.10g). The leaf anatomy in this species was very similar to that in Sect. Montanae in many respects. These included thick schlerenchyma girders, a restricted outer bundle sheath and the general shape of the leaf section and the air spaces. The main differences occurred in the midrib region where the schlerenchyma girders were less strongly developed and there was a small group of translucent cells forming a hypodermal layer below the bulliform cells.

4) Sect. Paniceae

Two species were examined - C.panicea and C.vaginata (Figs.5.5e,f; 5.8e,f; 5.11c,f). The two species showed several differences in leaf anatomy. The most obvious was in the number of vascular bundles in the leaf, but other characters such as the papillate abaxial epidermis in C.panicea and differences in the adaxial girders and the outer bundle sheaths also distinguish the species. A hypodermal layer of translucent cells was present in C.panicea and the bulliform cells in this species were larger. Once again, the schlerenchyma girders tend to be shorter and thicker than those found in Sect. Vesicariae and the air spaces are relatively smaller.

5) Sect. Spirostachyae.

One species was examined - C.binervis (figs.5.5j; 5.8a; 5.11a). This species differs from the Vesicariae in its short, wide broadly baculiform or securiform adaxial girders and the restriction of the outer bundle sheath to the region immediately around the bundle. Many of the smaller bundles were not attached to either epidermis by girders.

6) Sect. Flavae

One species was examined - C.extensa (Figs.5.5a; 5.8c; 5.11g). This species had a very distinctive leaf anatomy, with a crescentiform or faintly V-shaped section and an adaxial epidermis which consisted entirely of large swollen cells similar to the bulliform cells of most Carex species. There was no clear group of differentiated bulliform cells in this species. Many of the smaller vascular bundles were not attached to either epidermis by schlerenchyma girders while the girders in the larger bundles consisted of a small cap and a separate triangular strand below the epidermis. The air spaces tended to be rather small.

7) Sect. Elatae.

One species examined - C.laevigata. (Figs. 5.5a; 5.8b; 5.11b). The leaf anatomy of this species was very similar to that of C.binervis. The main differences appeared to the the slightly flatter leaf and smaller air spaces of C.laevigata.

8) Sect.Glaucae.

One species examined - C.flacca. (Figs.5.5c; 5.7g; 5.10h). C.flacca showed a rather distinctive regular arrangement of the vascular bundles in the leaf combined with broad, well developed schlerenchyma girders (broadly T-shaped to securiform). The abaxial epidermis was strongly papillate and the air spaces comparatively small.

9) Sect.Ammoglochin.

One species was examined - C.arenaria (Figs.5.4h; 5.8i; 5.11h). The leaf anatomy of this species was very distinctive with a narrow, thick V-shaped leaf. Most of the smaller vascular bundles were attached to the adaxial epidermis by baculiform girders but had no abaxial girders. They were usually situated nearer the adaxial surface than the larger bundles but were in the same orientation so there is no evidence for production of two rows of bundles by infolding of the leaf margin (cf. Metcalfe, 1971). A few large transparent cells were present below the bulliform cells but these did not form a distinct hypodermal layer. The outer bundle sheath was restricted to the area immediately around the vascular bundle.

10) Sect.Paniculatae.

One species was examined - C.paniculata (Figs.5.4g; 5.8j; 5.11i). The leaf section was thinly crescentiform and the bulliform cells were not strongly differentiated from the remainder of the adaxial epidermis which tended to have rather enlarged cells. This structure was

Fig. 5.17 Other sections.

a) C.acutiformis

b) C.pseudocyperus

c) C.hirta

Fig.5.11 Other sections - cont'd

f) C.vaginata g) C.extensa

h) C.arenaria i) C.paniculata

Fig. 5.12 Structure of stomata. (T/S) (x 650)

a) C.rostrata b) C.aquatilis

c) C.paupercula d) C.flacca

e) C.saxatilis f) C.lasiocarpa

Figs.5.13-5.15 T/S culms (x 30)

Fig.5.13 Section Vesicariae

a) C.rostrata b)-c) C.rostrata (broad-leaved forms)

d)-f) C.rostrata var.utriculata

Fig.5.14 Section Vesicariae (cont'd)

a) C.vesicaria b)&d) C.saxatilis

c)&e) C.grahamii (from Meall nan Tarmachan)

f) C.grahamii (from Glen Clova)

Fig.5.15 Other sections

a) C.hirta b) C.lasiocarpa

c) C.pseudocyperus d) C.acutiformis

e) C.flacca f) C.caryophyllea

g)-h) C.aquatilis

Figs.5.16-5.17 Epidermal structure from cellulose acetate impressions. (x 225)

ad. adaxial ab. abaxial

Fig.5.16 Section Vesicariae

a) C.saxatilis

b) C.grahamii (from Meall nan Tarmachan)

c) C.grahamii (from Glen Clova)

cf. also figs. 8.8 & 8.11.

Fig. 5.7 Other Sections.

a) C.hirta
b) C.lasiocarpa
c) C.pseudocyperus
d) C.lupulina
e) C.grayii
f) C.acutiformis
g) C.flacca
h) C.paupercula
i) C.caryophyllea
j) C.pilulifera

Fig. 5.8 Other Sections.

a) C.binervis
b) C.laevigata
c) C.extensa
d) C.aquatilis
e) C.panicea
f) C.vaginata
g)-h) C.aquatilis
i) C.arenaria
j) C.paniculata

Figs. 5.9-5.11 T/S median vascular bundle (x200)

Fig.5.9 Section Vesicariae

a)-b) C.rostrata
c)-d) C.rostrata var. utriculata
e)&g) C.grahamii (from Glen Clova)
f) C.grahamii (from Meall nan Tarmachan)
h) C.saxatilis (from Labrador)
i)-j) C.saxatilis

Fig.5.10 Other sections.

a) C.lasiocarpa
b) C.acutiformis
c) C.pseudocyperus
d) C.grayii
e) C.lupulina
f) C.hirta
g) C.paupercula
h) C.flacca
i) C.pilulifera
j) C.caryophyllea

Fig.5.11 Other sections

a) C.binervis
b) C.laevigata
c) C.panicea
d)-e) C.aquatilis

figs. 5.3 - 5.17

Figs. 5.3-5.5 T/S leaf in various Carex species (x 30)

(only half of each lamina is shown)

Fig. 5.3 Section Vesicariae

a)-b) C.rostrata

c)&e) C.rostrata var. utriculata

d) C.rostrata (broad-leaved form from western Ireland)

f) C.vesicaria

g)-h) C.saxatilis

i) C.saxatilis (specimen from Labrador)

j) C.grahamii (from Meall nan Tarmachan)

k)-l) C.grahamii (from Glen Clova)

Fig. 5.4 Other sections.

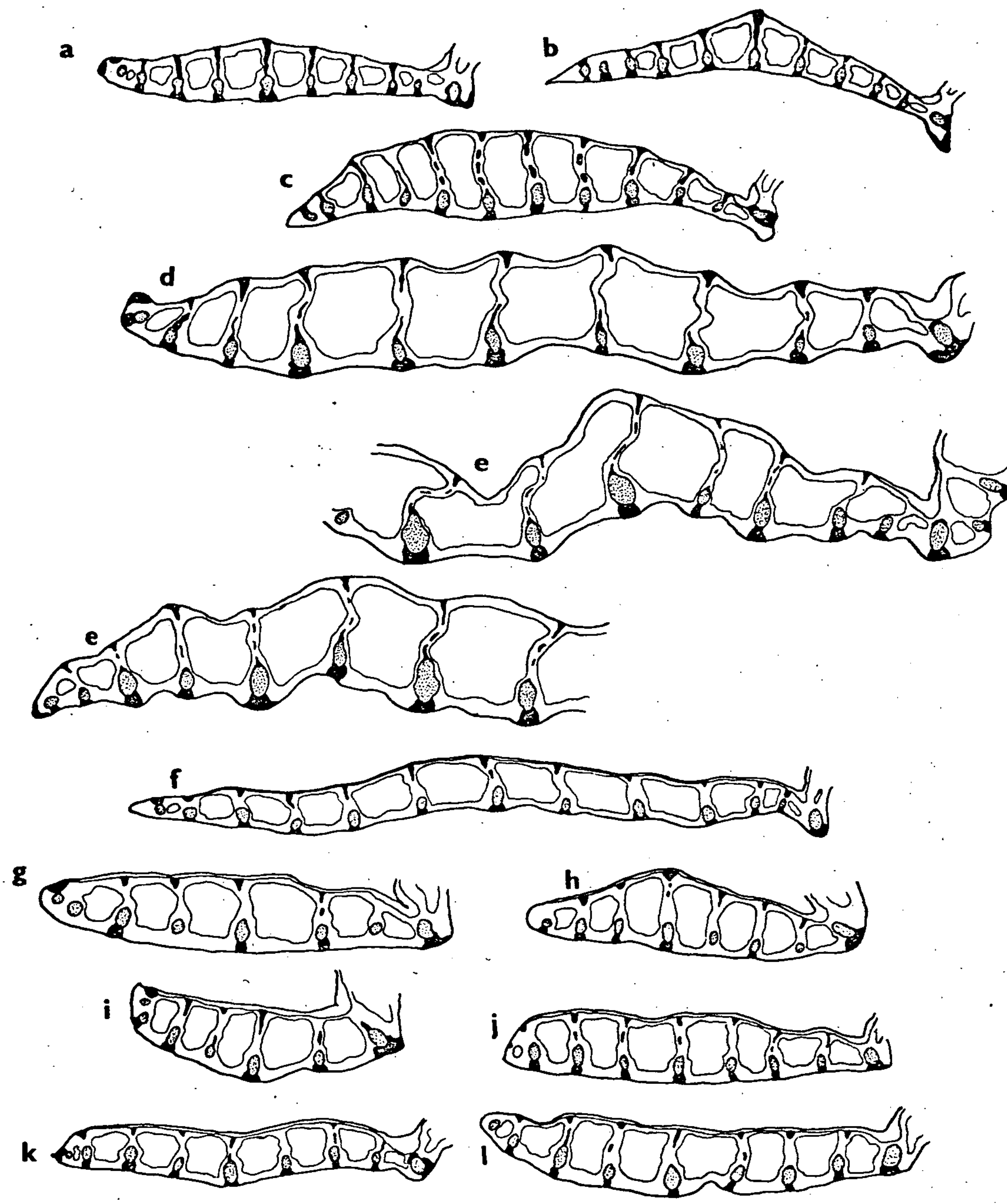
a) C.grayii
b) C.lupulina
c) C.hirta
d) C.lasiocarpa
e) C.pseudocyperus
f) C.acutiformis
g) C.paniculata
h) C.arenaria

Fig. 5.5 Other sections.

a) C.extensa
b) C.pilulifera
c) C.flacca
d) C.caryophyllea
e) C.vaginata
f) C.panicea
g) C.paupercula
h)-i) C.aquatilis
j) C.binervis
k) C.laevigata

Figs.5.6-5.8 T/S secondary vascular bundles (x200)

a)-b) C.rostrata
c)-d) C.rostrata var. utriculata
e)-f) C.grahamii (from Glen Clova)
g) C.grahamii (from Meall nan Tarmachan)
h)-i) C.saxatilis
j) C.saxatilis (from Labrador)



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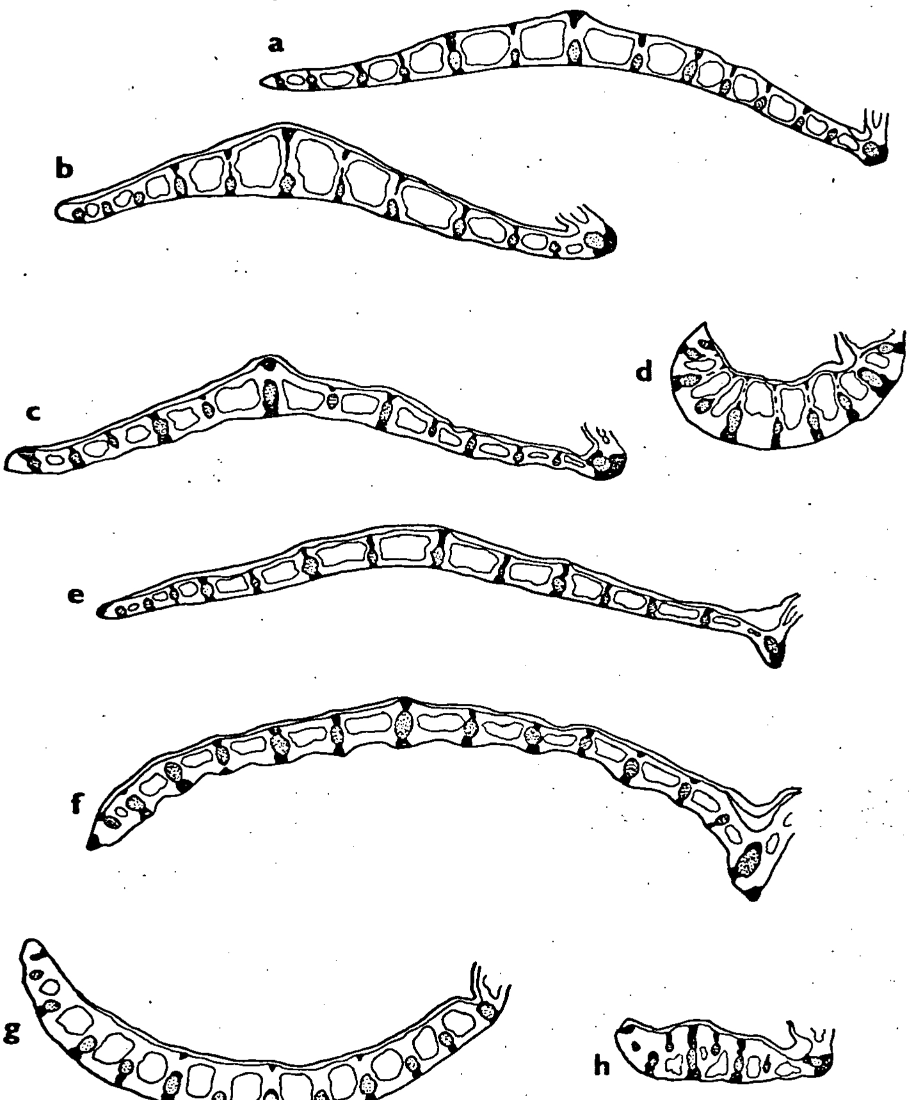

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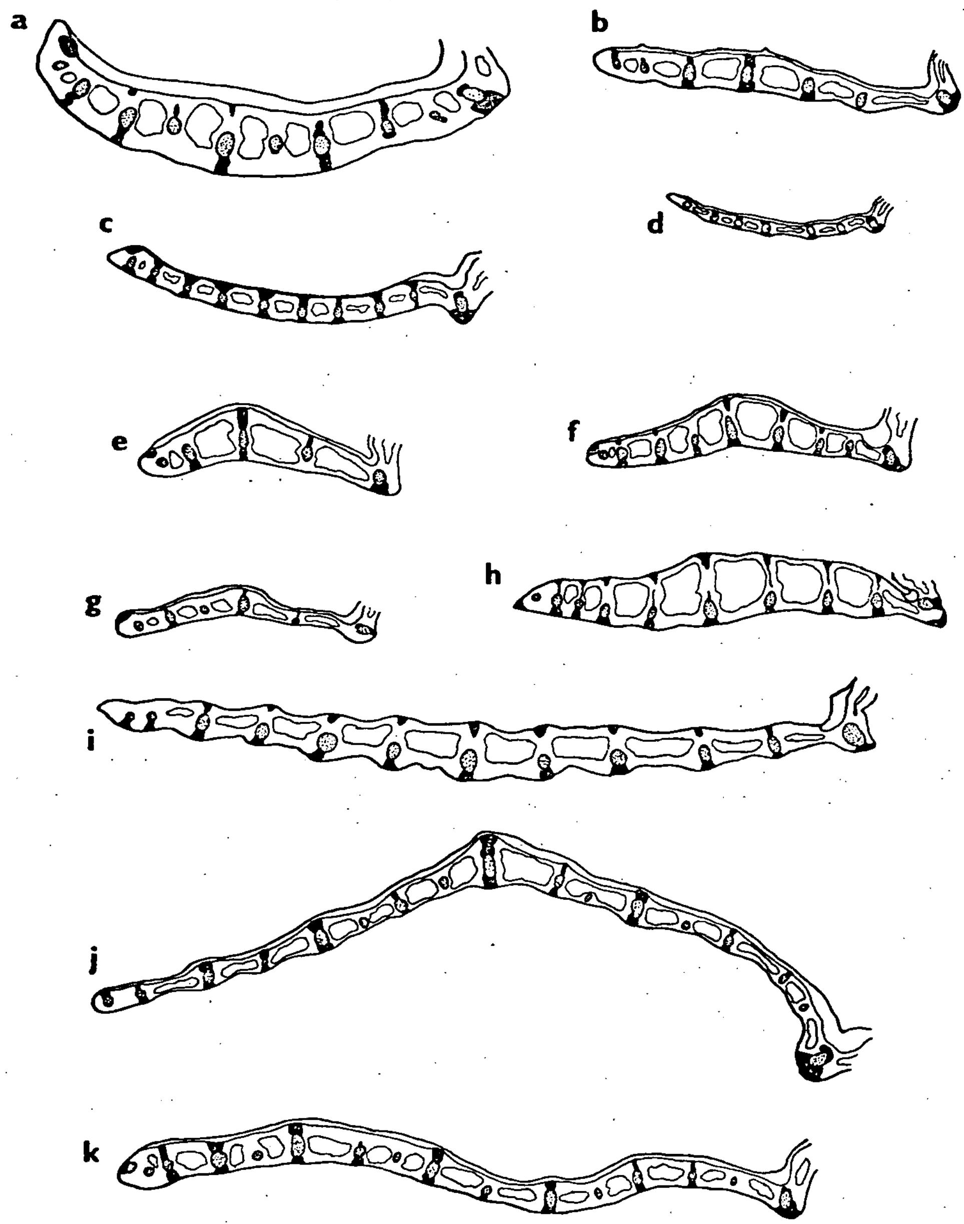

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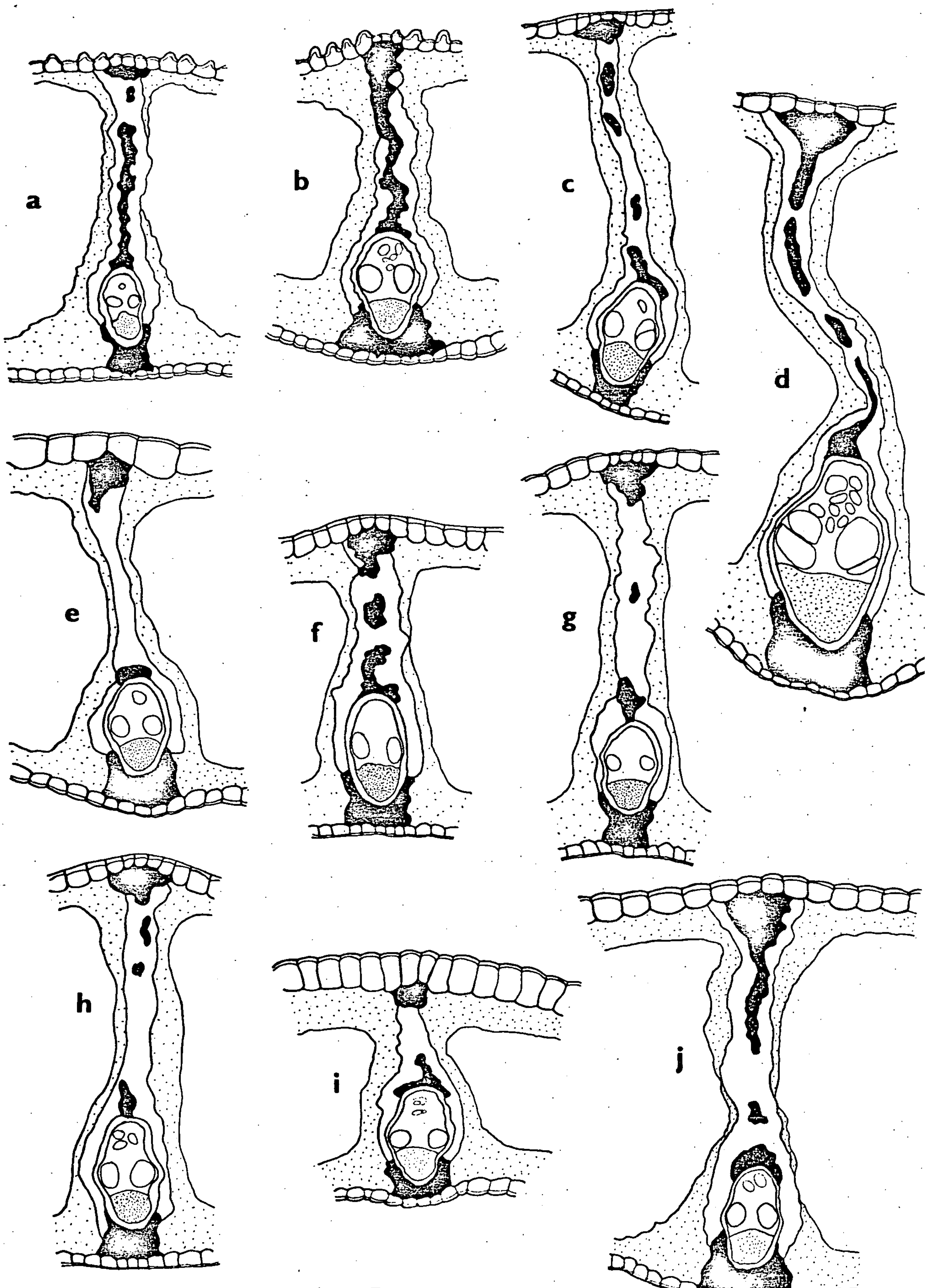

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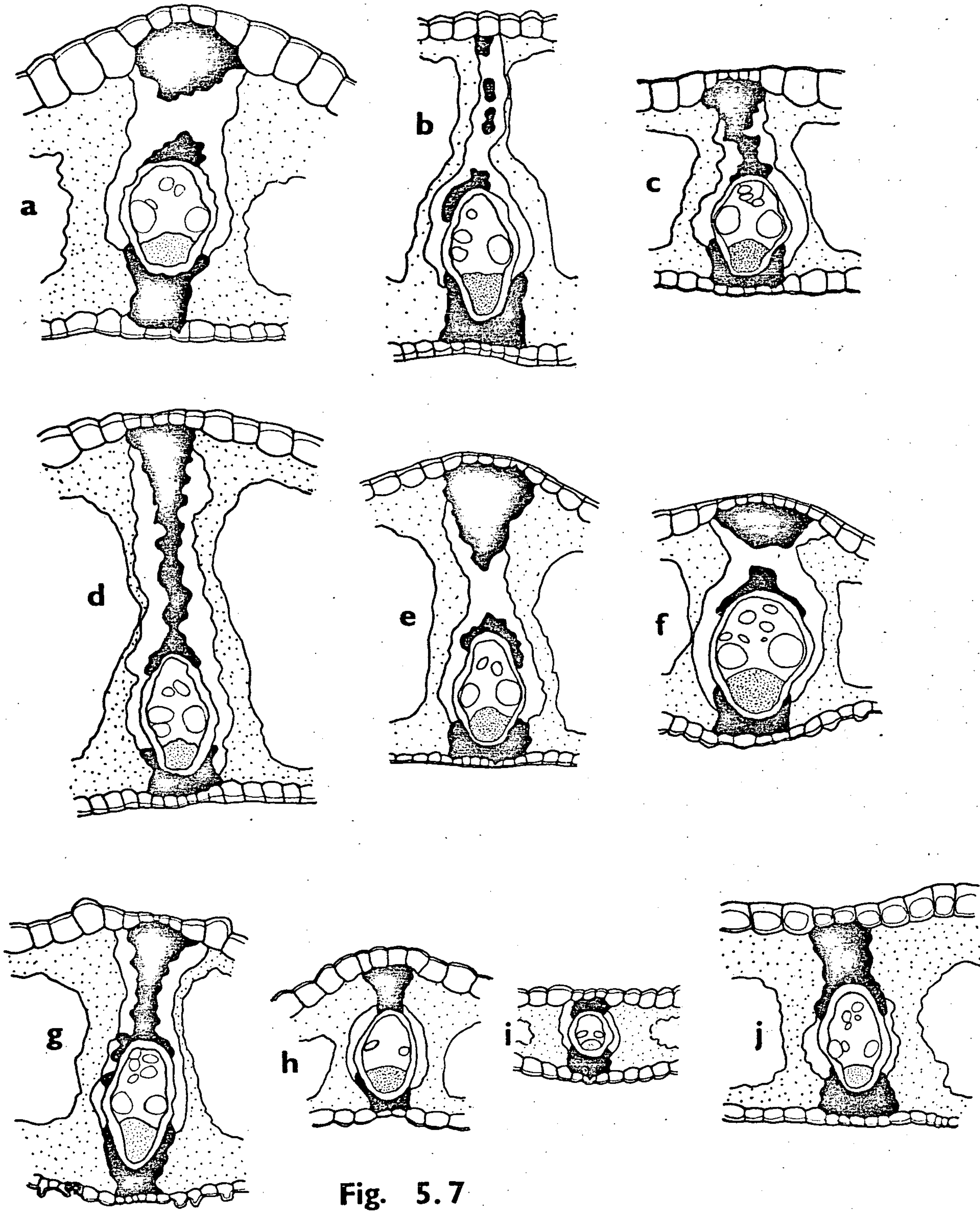

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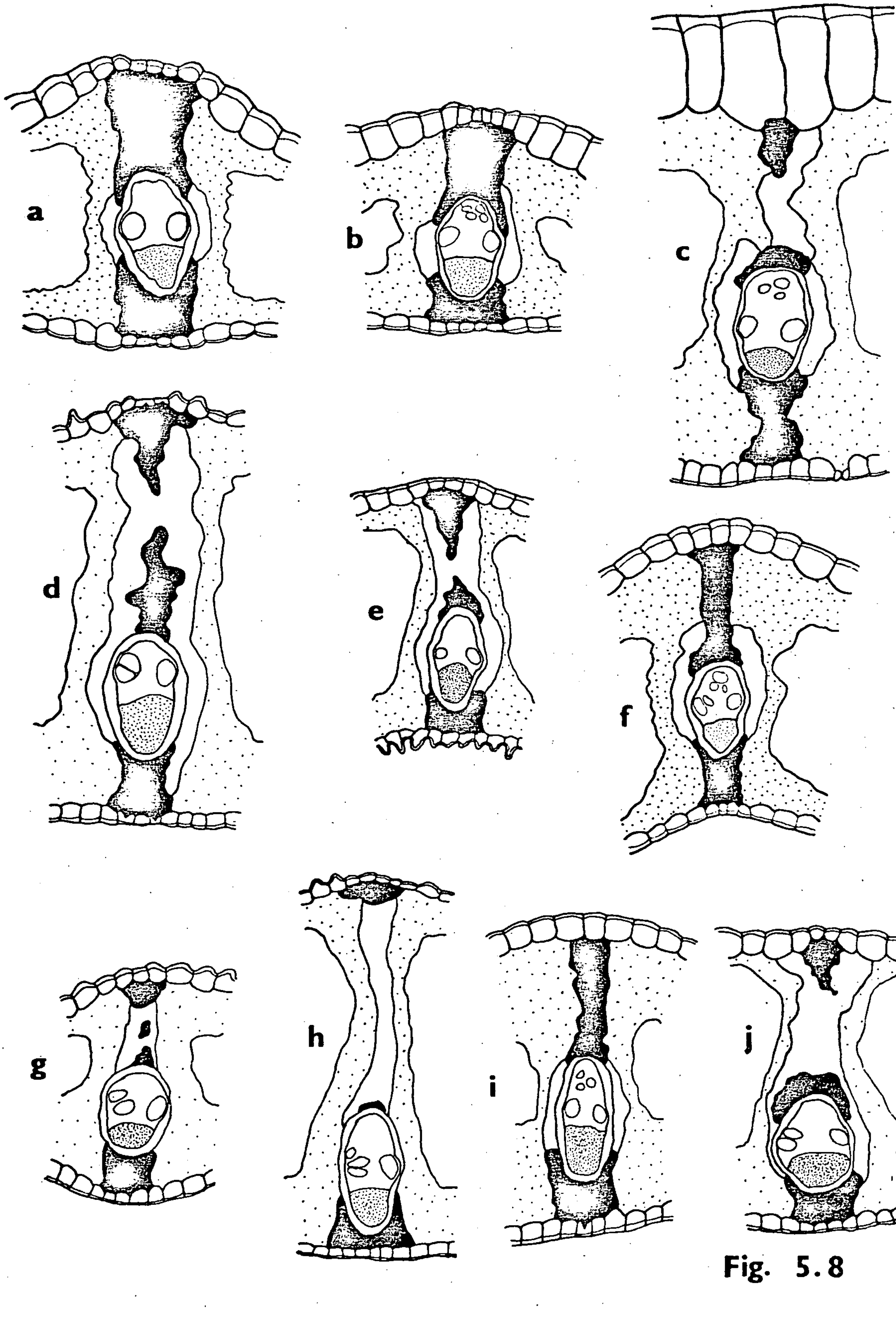

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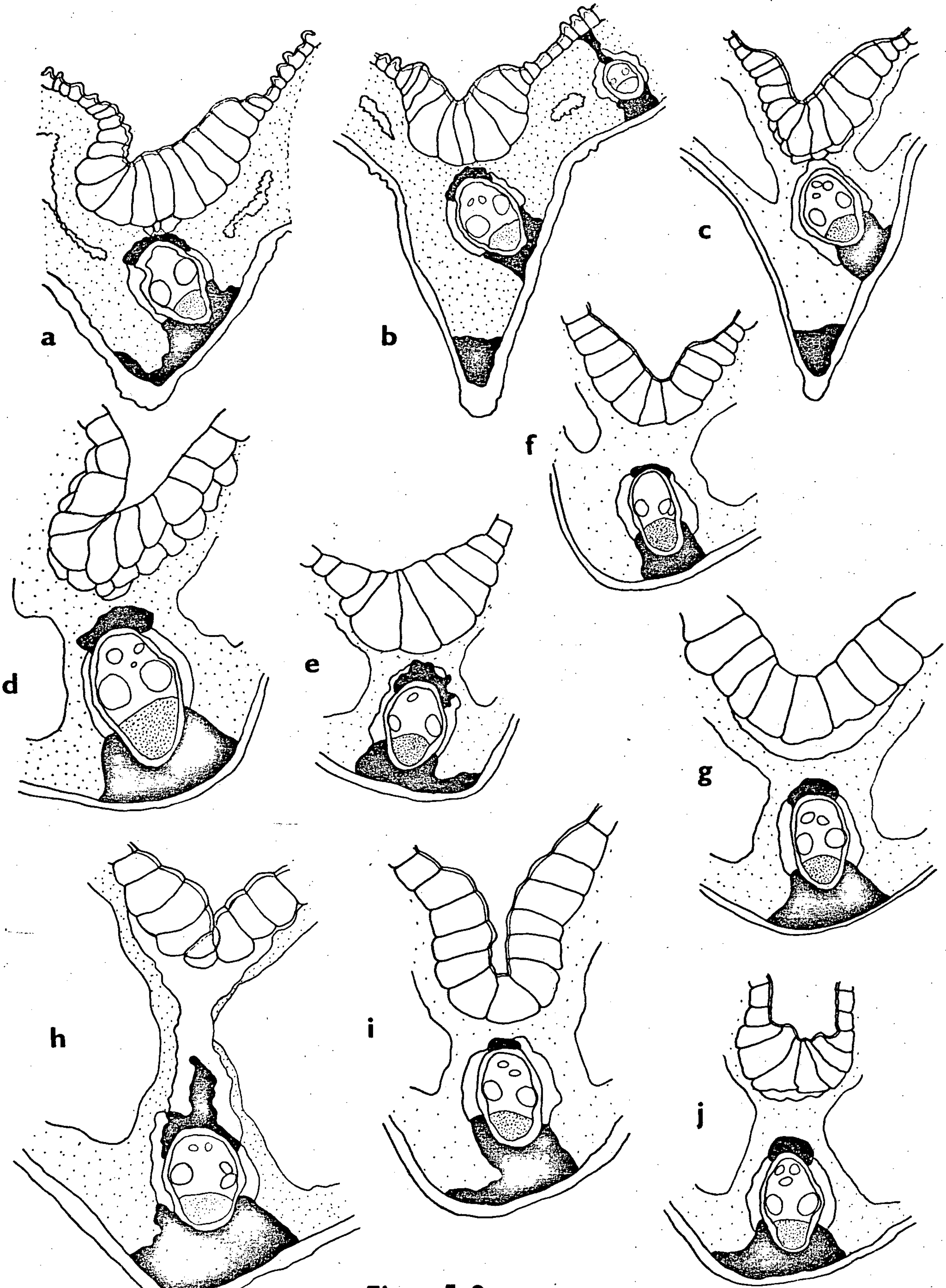

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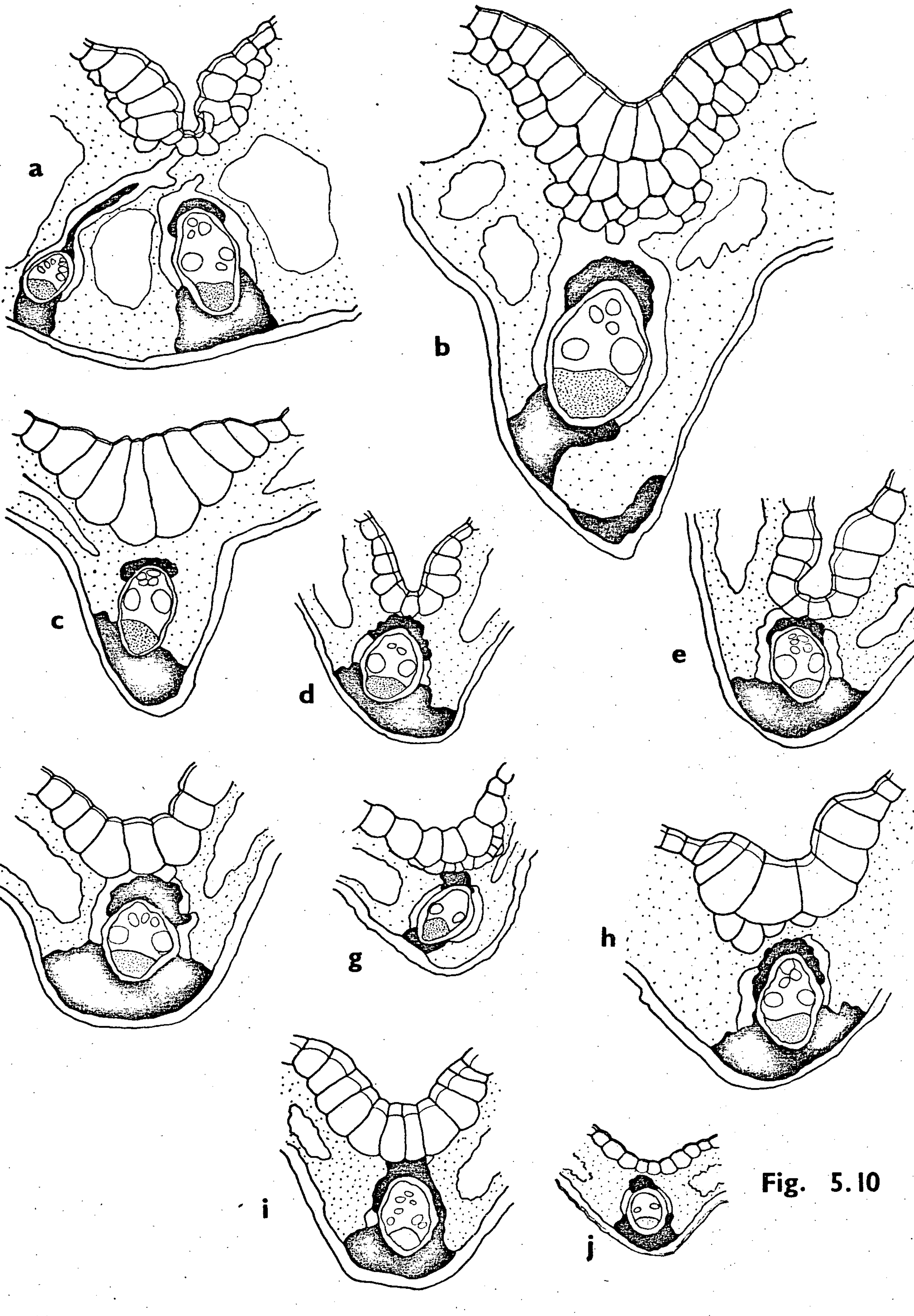

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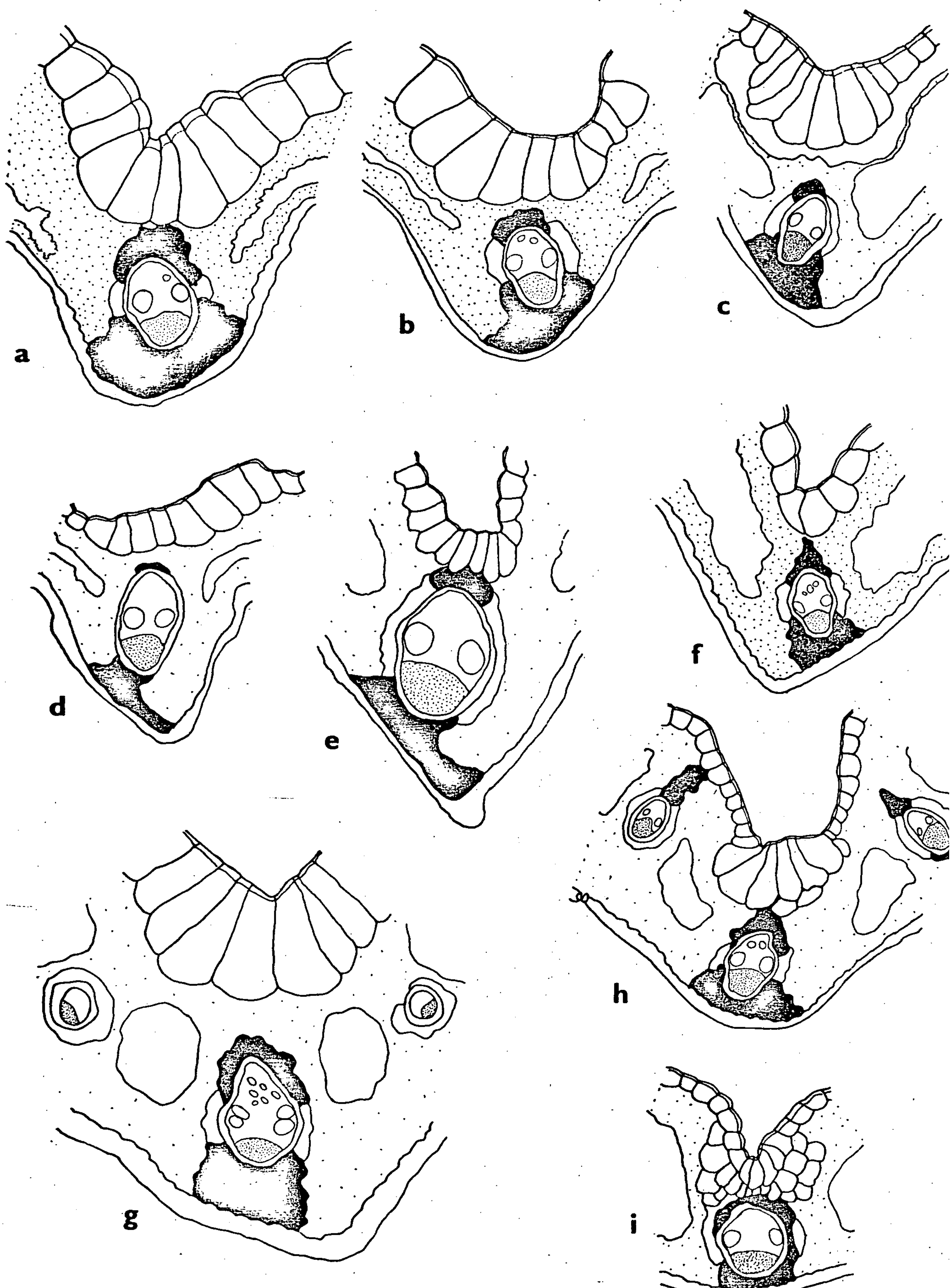

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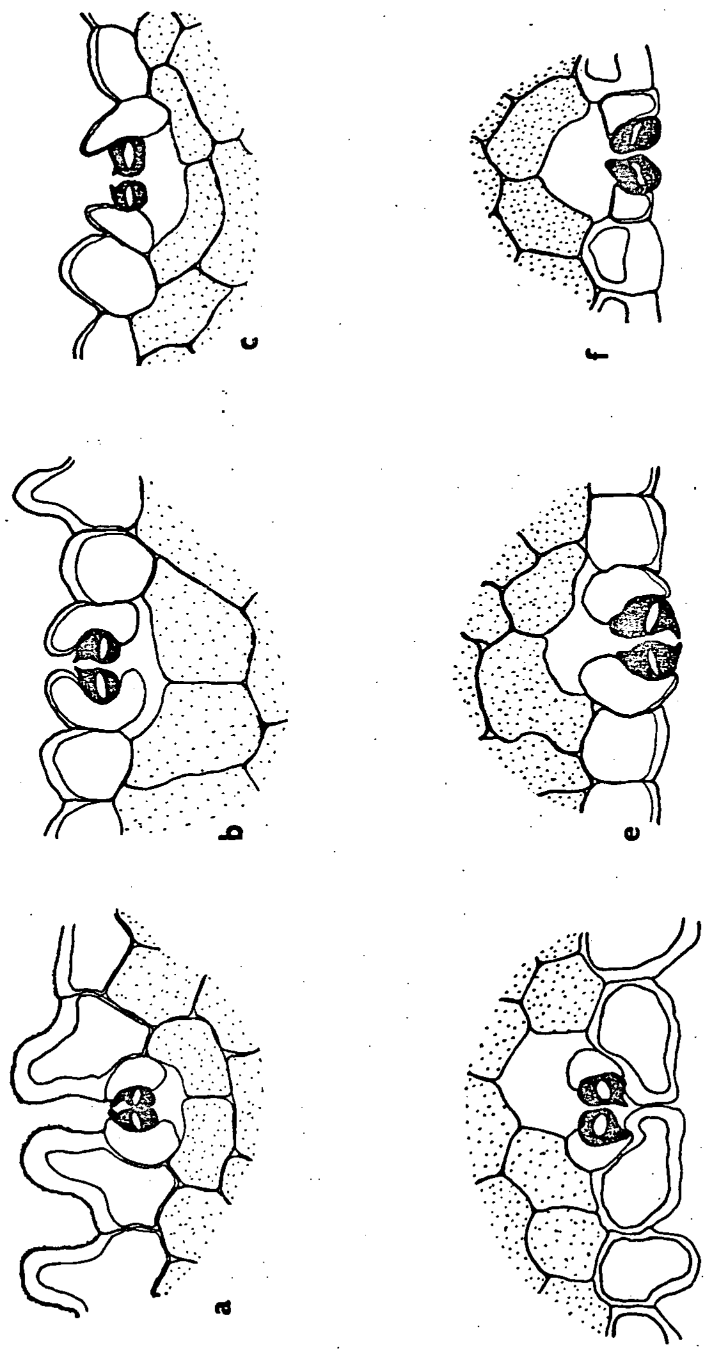

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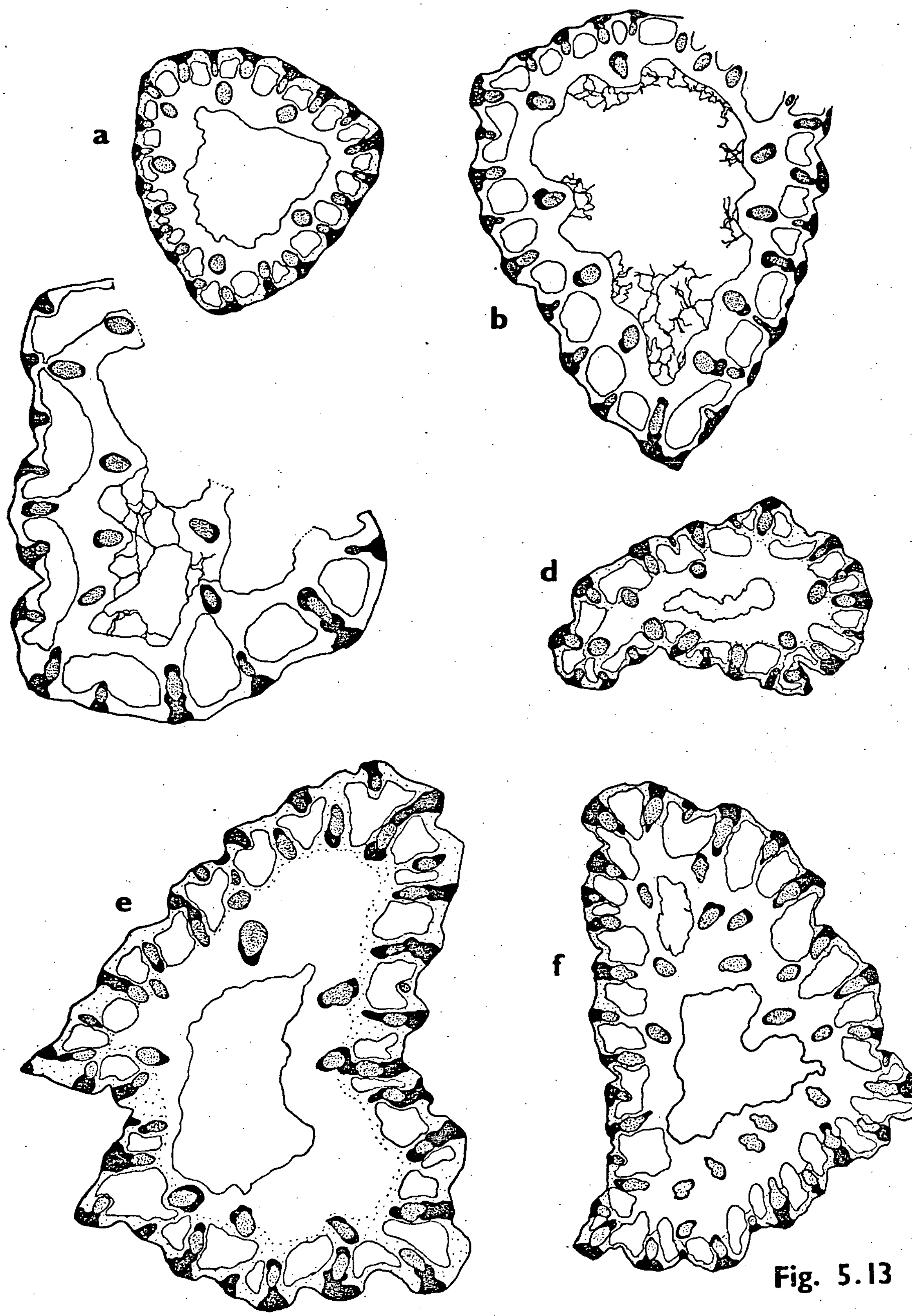

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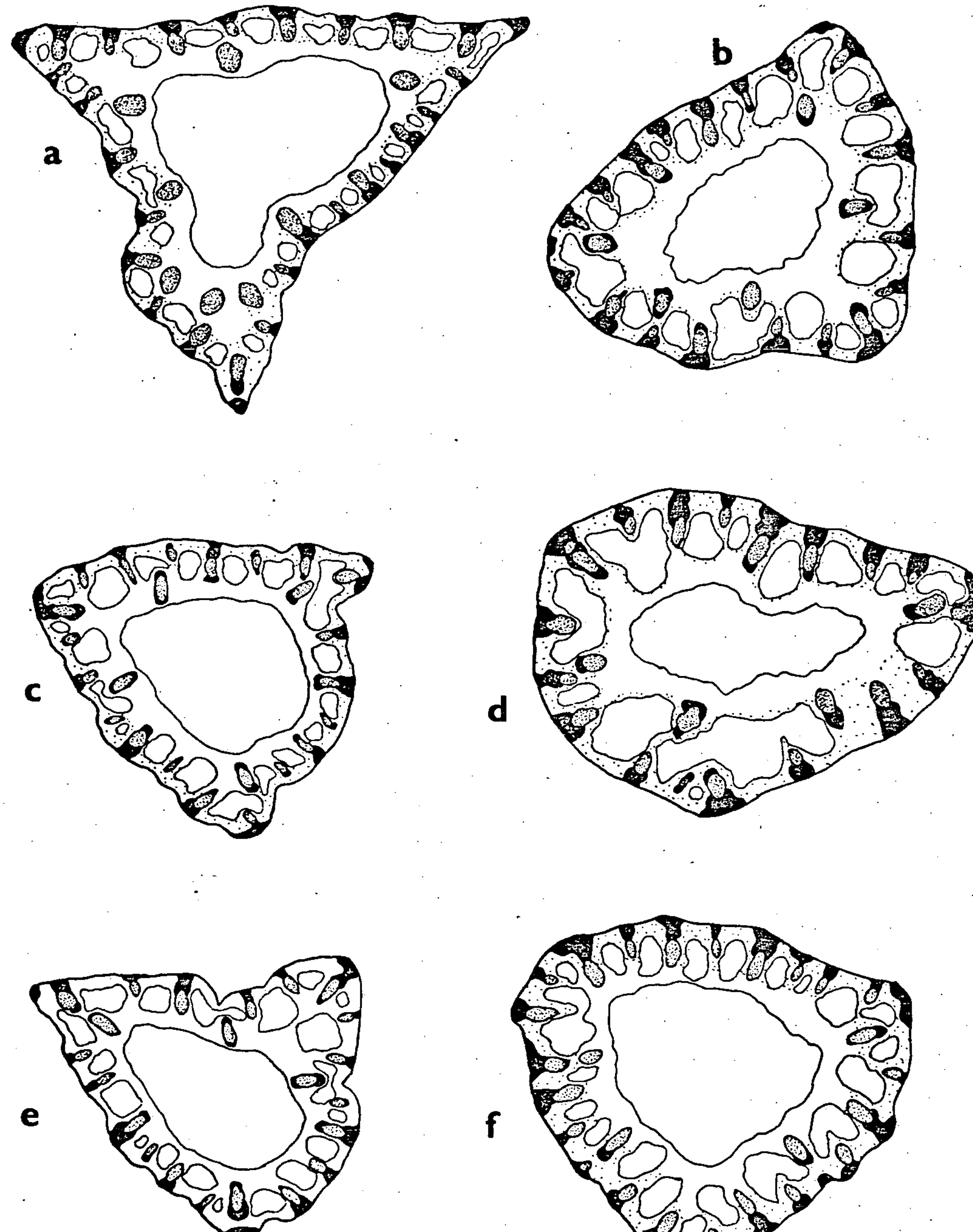

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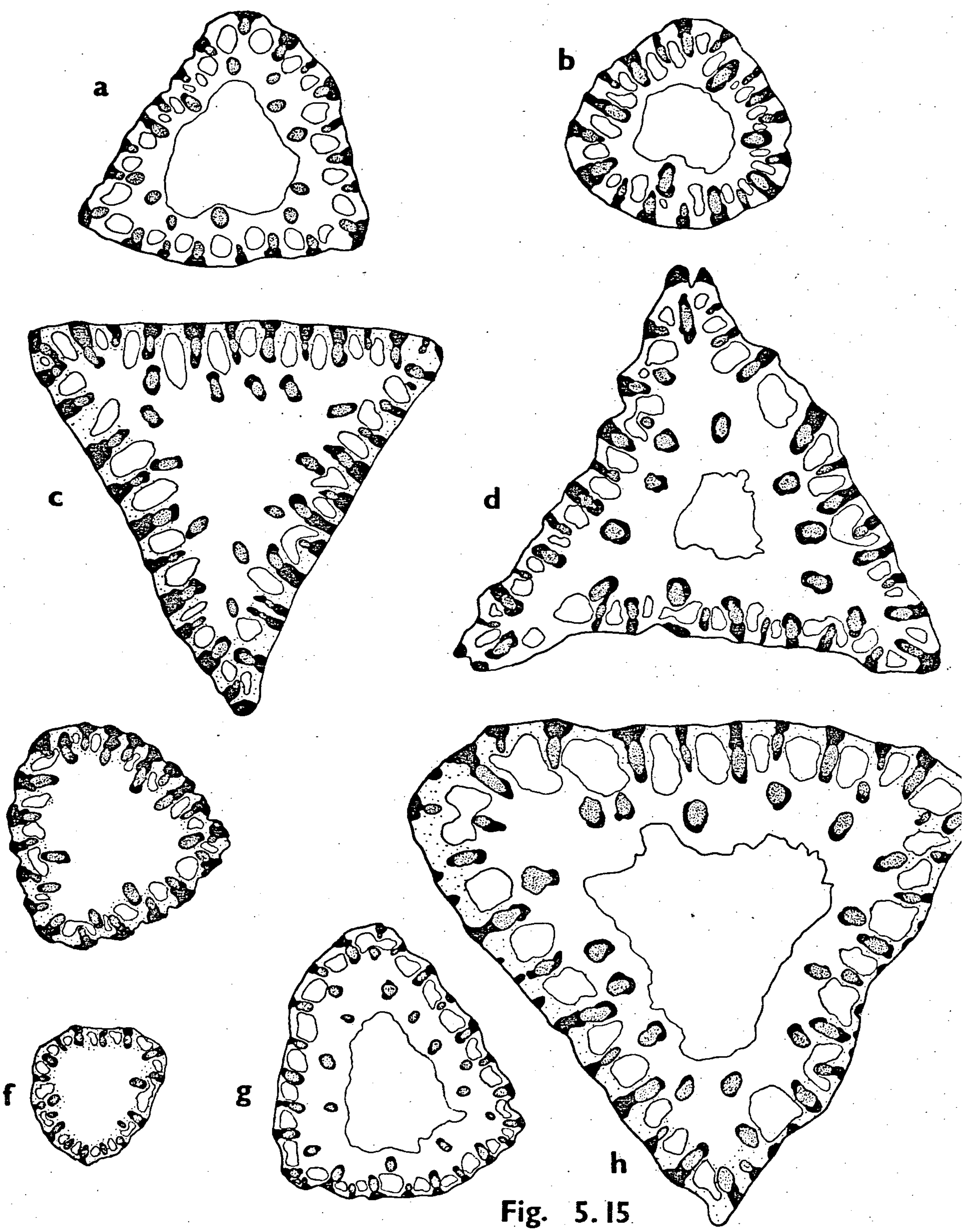

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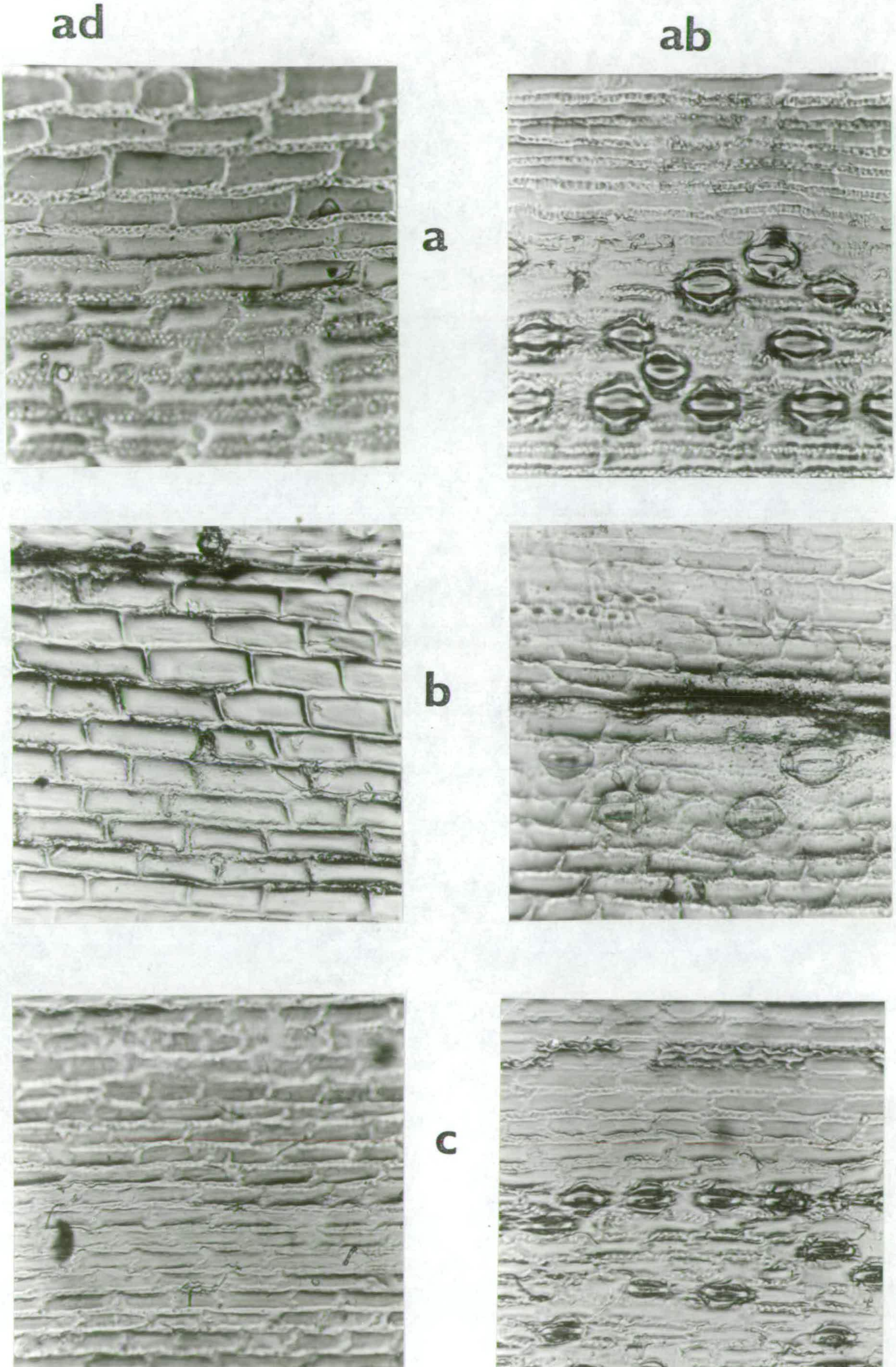
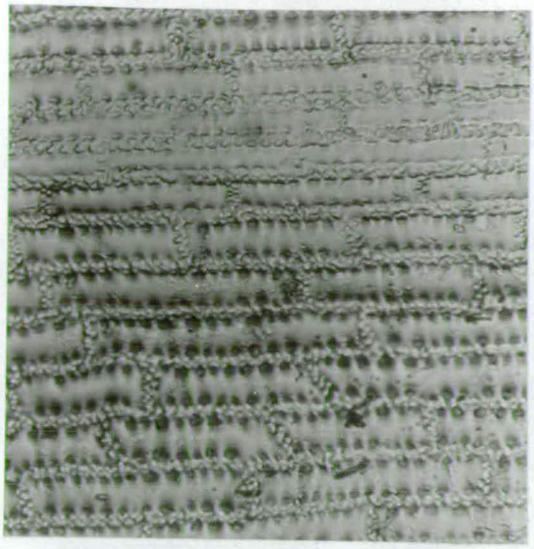


fig. 5.16

ad

ab



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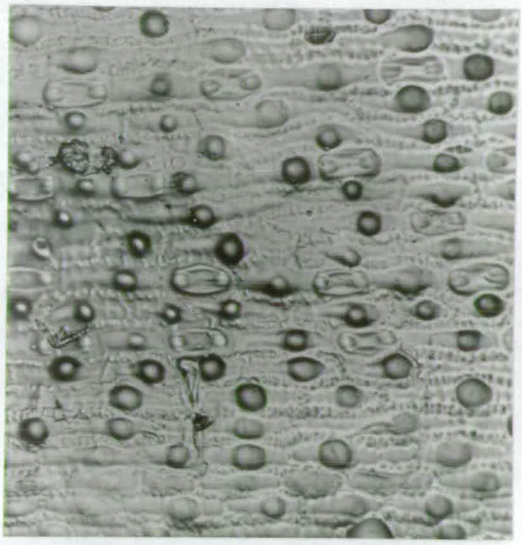

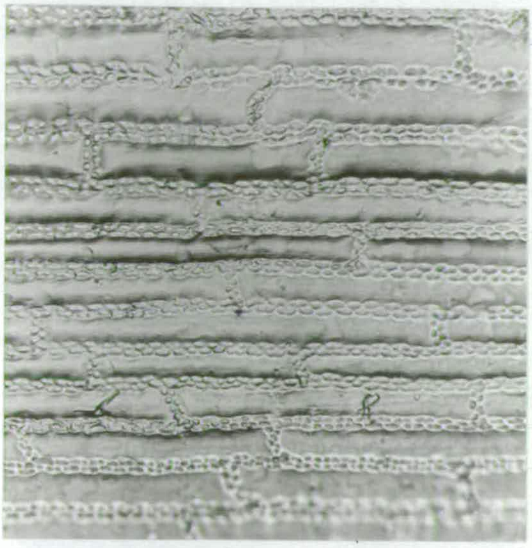

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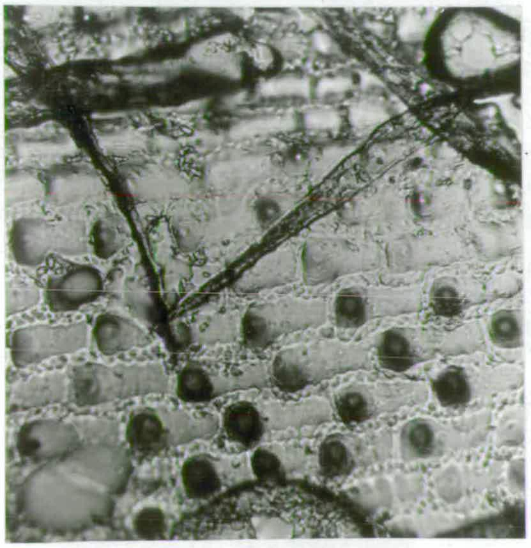

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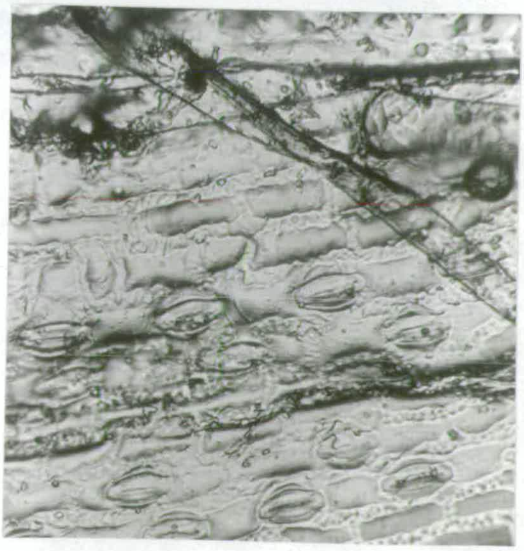


fig. 5.17

similar to that in C.lasiocarpa and C.extensa but did not occur in such an extreme form. A prominent mass of translucent cells occurred below the bulliform cells.

5.6 Discussion and Conclusions

The primary aim of the anatomical work described in this chapter was to examine the possibility of using anatomical data to aid delimitation of sections within Carex. A set of characters which was common to all the members of a section but was absent or rare in the remaining sections would strongly support the idea that the species included in the section form a natural group, although the possibility of convergence can rarely be completely ruled out. Non-correlation of anatomical and morphological character sets may indicate either that the anatomical characters are conservative, and faster evolutionary change and convergence has occurred in morphological characters, or that evolutionary change in anatomical characters has proceeded at a faster rate, allowing anatomical divergence to occur in a group which is still morphologically uniform. If the first alternative was applicable the sections would be polyphyletic, while the second would imply that they are monophyletic. Differing evolutionary rates are, of course, well known in morphological characters (cf. Davis & Heywood, 1963; Sneath & Sokal, 1973). With the possible exception of certain features of wood anatomy (cf. discussion in Davis & Heywood, op.cit.) there is no evidence to suggest that anatomical characters give a clearer indication of cladistic relationship than standard morphological attributes and they must therefore be treated as a source of additional information which receives no more weighting than normal morphological characters. To assess the value of anatomical characters, it is first necessary to decide whether or not they agree with morphological evidence, and then if they do not, whether they can provide the basis for a more satisfactory classification system.

The members of Sect. Vesicariae appear to form a more or less distinct group on the basis of leaf anatomy, but there are considerable problems in drawing up a list of specific characters which can be used to define the group. The most obvious differences separating this section from the rest of the genus appears to be the rather tall, slender schlerenchyma girders, or if these are absent or broken into separate strands the tall thin column formed by the outer bundle sheath and a very thin layer of chlorenchyma (usually only one or two cells thick). The air spaces are usually large in comparison with those found in many other sections. These observations also seem to apply to several species not examined in the present survey, since the illustrations given by Akiyama (1935, 1942) for C. laevirostris and C. rotundata generally conform, although the air spaces in the latter appear to be smaller than is usual in the Vesicariae. Numerous differences between species also occur. C. rostrata is especially distinctive in possessing a strongly papillate adaxial epidermis with adaxial stomata, characters which have not been found in any other member of Sect. Vesicariae. This combination appears to be rare in Carex as a whole, and in the present study C. aquatilis was the only other species found to possess it. It is also reported for C. nigra, C. montana and a few other species (Metcalfe, 1971), but I have not been able to confirm Metcalfe's report of adaxial papillae in C. flacca. C. rotundata appears from Akiyama's illustration to have a more or less crescentiform leaf section with ill-defined bulliform cells and somewhat resembles European material of C. lasiocarpa. There is also considerable intraspecific variation as can be seen from the comments on C.rostrata. A hypodermal layer of translucent cells was present in some specimens of C.rostrata (mainly in wider leaved material) but the development of this character is very variable in the Vesicariae. Hypodermal layers are also reported for C.laevirostris & C.saxatilis (Akiyama, 1935) and in C.rotundata (Akiyama, 1942), but most of the specimens of C.saxatilis examined in the present survey had no hypodermal layer, except the one from Labrador. In spite of the variations within species, and within the section, however, the Vesicariae appear to be relatively homogeneous in their anatomy. Le Cohu (1970) has suggested that, on the basis of anatomical evidence, Sect.Vesicariae should be split into one section containing C.rostrata and another containing the remaining species. She places heavy emphasis on the presence of more than two vascular bundles in the utricle of C.vesicaria while C.rostrata has only two, and also emphasises the differences in epidermal characters between the two species. In her view, the morphological resemblances between the species are the result of convergence between species growing in similar habitats. It seems that this suggestion cannot be entertained, however, since the use of such characters to split Section Vesicariae would lead to considerable difficulties. In particular, the position of C.rostrata var.utriculata would be very doubtful. This North American form is distinguishable from the European type variety only with difficulty on morphological grounds (cf. Ch.8), but it has more than two vascular bundles in the utricle and also has a smooth epidermis with abaxial stomata, i.e. its anatomy closely resembles that of C.vesicaria. The presence of a band of prickle cells above the insertion of the ligule closely resembles C.rostrata, however, and the confusion is further added to by the presence of more than two vascular bundles in the utricles of broad leaved forms of C.rostrata from Western Ireland (although this point requires to be examined in more detail). The latter plants are similar to typical C.rostrata in epidermal characters. Although it is possible that C.rostrata var.utriculata should be considered as a separate species, it would seem to be impossible to split Sect.Vesicariae on the grounds suggested by Le Cohu without including elements of C.rostrata in the same section as C.vesicaria. In view of the biosystematic and cytological evidence, it seems preferable at present to retain the commonly accepted circumscription of Sect.Vesicariae.

The other sections investigated show varying degrees of homogeneity. Members of Sect.Paludosae generally have a series of characters which are shared by almost all of the species examined but are not found exclusively in this section. The presence of a more or less well developed hypodermal layer below the bulliform cells was found in C.acutiformis and is also reported for C.riparia (Mazel, 1891; Crawford, 1910; Le Cohu, 1967; Metcalfe, 1971) and in C.scabridifolia and C.pumila (Akiyama, 1935) and C.trifida (Herriot, 1905). All of these species tended to have rather shorter, thicker schlerenchyma girders and smaller air spaces than was typical in the Vesicariae. Two species were papillate (C.riparia and C.acutiformis - both with abaxial papillae) while the remainder were non-papillate. Sect.Montanae likewise shows a series of similar leaf structures in C.caryophyllea and C.pilulifera and a similar set is reported for C.montana (Le Cohu, 1967) and C.ericetorum (Crawford, 1910), with thin leaves, broad girders and bulliform cells which are not very strongly developed. Members of Sect.Hirtae, in contrast, vary considerably in their anatomy. C.lasiocarpa is very distinctive with its crescentiform leaf section, ill-defined bulliform cells and the presence of a hypodermis. The other members of the section are more uniform in appearance, e.g. C.hirta (present study), C.ligulata, C.myabei and C.drymophila var. akinensis (Akiyama, 1935), and it is interesting that the figure given by this author for C.lasiocarpa var. occultans shows virtually none of the peculiarities seen in European material as it has prominent bulliform cells and a slightly flanged V-shaped section. C.hirta is, however, distinctive in its numerous unicellular hairs and adaxial papillae. C.lasiocarpa requires more detailed study before any definite conclusions can be reached on the significance of the leaf anatomy of the European variety. With regard to Sect. Pseudocypereae, it is unfortunate that at present, only two species have been examined and, of these, the only one illustrated has been C.pseudocyperus (present study; Mazel, 1891; Crawford, 1910; Le Cohu, 1967; Metcalfe, 1971). Akiyama (1942) unfortunately gives only a description (in Japanese) for C.capricornis and it is therefore not possible to survey the anatomy of this section with any confidence.

It would appear from the above that sections within the Vesicariae complex and many of the other sections, in subgenus Carex at least, do tend to show some degree of homogeneity in their anatomy, but many characters do not follow sectional delimitation at all closely. The general conclusion to be drawn would appear to be that anatomical evidence is to a certain extent correlated with morphology, but considerable evolution of anatomical characters within sections must have occurred. In particular epidermal characters such as the presence or absence of papillae and the position of the stomata vary considerably within most sections. and in some, almost all the possible character combinations are found. Any attempt to establish sections on the basis of these characters would lead to the production of groups which are, on morphological grounds at least, very heterogeneous. If the above conclusions are correct, however, it would appear that there is some justification for the use of anatomical characters to support morphological data in deciding whether or not to merge sections as suggested in Chapter 1. To what extent therefore does the anatomical evidence support merging of the sections of the Vesicariae complex? The answer to this question seems to be that anatomy generally provides very little evidence for combining these sections into a single unit. Sects. Paludosae, Hirtae and, as far as can be determined at present, Pseudocypereae, while similar in their general anatomy to Sect. Vesicariae have some distinctive characters of their own which suggests that they should probably be maintained as separate sections. This does not imply that they are not to some extent related, only that there are sufficient differences between them to make merging of the sections a rather dubious procedure without compelling evidence from other sources. The differences between these sections appear to be at least as large as those separating them from members of Sects. Flavae or Spirostachyae. On the whole, the anatomical evidence supports the present delimitation of sections, but it must be emphasised that the characters used to separate sections are for the most part rather ill-defined and individual characters are not confined exclusively to single sections. On both morphological and anatomical grounds, the sections of Carex are generally polythetic taxa (cf. Sneath & Sokal, 1973) and there are few characters which are confined to single sections. Considerable variation also occurs within sections and it would be extremely difficult to produce a useful and consistent classification on the basis of anatomical characters alone. One of the major problems in both the present study and in previous investigations on anatomy in Carex is that the sampling so far carried out has been very limited. This is of course inevitable in an enormous genus with as many as 2,000 species, but it is still true that in many sections only a very small proportion of the species included by Kükenthal have been studied. It is likely that more detailed surveys of small groups and single sections might provide a very useful basis for more extensive surveys. Both Mazel (1891) and Akiyama (1942) concluded that anatomical data were generally of little value in establishing a classification above the species level in Carex, but their samples from each section were usually very small. When data from these two studies are combined with those of the present survey as well as those of Metcalfe (1971) and Crawford (1910), there is some evidence to suggest that anatomy may be to some extent significant, but a thorough assessment of the value of anatomical characters must await a more detailed survey which includes a higher proportion of the species within each section. A review of the published data would be extremely interesting, but this would be a major undertaking. At species level, however, there is little doubt that anatomy, and epidermal characters in particular, may be extremely valuable in helping to distinguish between morphologically closely related forms.

CHAPTER 6 CYTOLOGY

6.1 Introduction

This chapter describes the rather limited cytological study carried out on members of the *Vesicariae* complex, and summarizes the published information on chromosome numbers for this group. No detailed discussion on the cytology of *Carex* is offered since this has already been done by Faulkner (1970), who reviews much of the literature on the cytological peculiarities of the genus, and presents evidence from his own work on Sect. *Acutae*. Löve *et al*. (1957) also give a good general introduction and a list of over 400 references, though a large proportion of these are concerned with taxonomic literature on Sect. *Capillares*.

Carex species have been used as cytological material by a number of investigators because they offer a number of interesting cytological problems, as do the majority of the *Cyperaceae*. These first became apparent when it was realised that in microsporogenesis, three of the four products of meiosis abort, and the remaining nucleus goes on to divide to form the pollen grain nuclei. The pollen mother cell is released intact as a pollen grain and does not divide. The pollen grains formed in this way have been variously described as cryptotetrads or pseudomonads (cf. Davis 1966). When a number of chromosome counts had been made for the genus, especially after Heilborn's surveys (1924, 1939), it rapidly became obvious that *Carex* offered an unrivalled example of an aneuploid series which showed no obvious signs of normal polyploidy although a wide range of numbers had been reported. This was in part explained by the observation that many of the members of the *Cyperaceae* possessed an unlocalised centromere rather than the normal single localised centromeres.

Unlocalised centromeres are common to both the Cyperaceae and the Juncaceae (incl. Juncus) and it is also found in a number of unrelated groups. Löve et al. (1957) state that this feature is found in the Conjugatae, Desmids, Plasmodiophorales, nematodes and certain insect groups, and that some related phenomena occur in the Zingiberaceae and Musaceae. A number of authors (eg. Cronquist, 1968) have claimed that this character is good evidence for a close relationship between the Cyperaceae and Juncaceae. Most of the detailed studies made on diffuse centromeres have been carried out in animal groups (cf. White, 1973) but in plants, it has been most intensively studied in Luzula (Juncaceae), and in particular, L.purpurea which has only 6 large chromosomes in its diploid complement (Malheiros & de Castro, 1947; Nordenskiöld, 1962). Eleocharis has also been investigated in some detail, and it is only in these two genera that there is more or less conclusive evidence of the diffuse centromere, derived from observations of the behaviour of chromosome fragments produced by X-ray irradiation (Nordenskiöld, 1962; Hakansson, 1958). In Carex, chromosome numbers tend to be rather high and the chromosomes themselves are very small, so that detailed investigation of morphology and behaviour is much more difficult than in Luzula and Eleocharis. As a result, there is less direct evidence of diffuse centromeric activity, but most of the observations made are consistent with holocentric centromeres, and there can be little doubt that this phenomenon is characteristic of the genus as a whole.

The presence of diffuse centromeres is very frequently accompanied by auto-orientation of the chromosomes at metaphase I followed by an equational first division, i.e. the bivalents lie on the metaphase plate with their two chromosomes side by side and sister chromatids separate at anaphase I. There appears to be a considerable body of evidence to suggest that auto-orientation occurs, at least in Luzula. Nordenskiöld observed the separation of heteromorphic bivalents in Luzula hybrids (Nordenskiöld, 1961) and the presence of relict chiasmata in L.purpurea (Nordenskiöld, 1962) and concluded that these were in agreement with the configurations to be expected from a combination of diffuse centromeric activity and auto-orientation. Observations on the fragments produced by X-rays also confirmed these points. Although auto-orientation does not necessarily accompany the presence of diffuse centromeres, since co-orientation has been found in some insects (White, 1973), it seems almost certain that it does occur in Carex, in view of the observations reported by Faulkner (1970), and those of other authors summarised by Faulkner.

The cytological peculiarities of the Cyperaceae described above have had a profound effect on karyotype evolution in this family. In genera such as Fimbristylis where the centromeres appear to be of the normal localised type, a fairly normal series of polyploids with regular numbers and only a few aneuploids is reported. Carex and Scirpus, by contrast, show extensive aneuploid series. It is interesting that some genera in the Cyperaceae have remarkably constant chromosome numbers - Uncinia has 2n = 88 for almost all of its species and in Fedorov (1969) only about three counts of other numbers are recorded. As mentioned earlier, Carex provides what is probably one of the most extensive aneuploid series in the Angiosperms, with the reported chromosome numbers ranging from 2n = 12 to 2n = 112 (Fedorov, 1969) and a wide range of intermediate numbers which do not follow any obvious polyploid series. A number of attempts have been made to find a basic number for the genus (e.g. Heilborn, 1939; Tanaka, 1949; Wahl, 1940) by looking for maxima in the distribution of numbers within the genus. However, as Faulkner (1972) points out, it is necessary to postulate several basic numbers, with secondary balancing above and below the strict polyploid multiples, brought about by aneuploidy. Under such conditions, the concept of a basic number seems to be rather meaningless when applied to the genus as a whole. It seems likely that several basic numbers exist in different sections, and as Löve et al. (1957) showed, it is possible to estimate the base number for a small section with some confidence. In this case, size differences in the chromosomes made it possible to distinguish true polyploids and also to estimate the original number in the section. Unfortunately, few other sections show such a clear-cut polyploid series and in most cases the situation is probably much more complicated.

One of the major consequences of the unlocalised centromere structure is that aneuploidy becomes much more likely, since any chromosome fragment can behave as a complete chromosome and is not lost as in the acentric fragments produced by organisms with localised centromeres. A simple splitting of the chromosome is all that is required to increase the number, instead of the complex sequence of breakage and translocation necessary in most other organisms (cf. Stebbins, 1950). It is extremely difficult to distinguish between the effects of simple auto- or allopolyploidy and multiplication of a large number of chromosomes by splitting. In a study of Luzula, Nordenskiöld (1951) found that cases of normal polyploidy occurred in which the chromosome number was doubled while their size remained constant. In other species, however, the size of the chromosomes decreased with increasing polyploid level and it was suggested that this type of polyploidy was brought about by splitting of all the chromosomes in the complement so that the number of chromosome bodies was doubled while the amount of genetic material remained constant. The name 'endo-nuclear polyploidy' was suggested for this phenomenon but it is very often referred to as agmatoploidy or pseudopolyploidy. It is not clear to what extend agmatoploidy has occurred in Carex, but the evidence available at present suggests that it is comparatively rare, and that small-scale aneuploidy is prevalent in the genus. Löve et al. (op.cit.) obtained a polyploid series in which the species at $2n = 18$, 36 and 54 had similar proportions of large and small chromosomes in their complements, while aneuploids at $2n = 38$, 40, 56 and 58 had more numerous small chromosomes, suggesting that the aneuploids had been formed by fragmentation of one or more of the large chromosomes in the complement. There appeared to be no evidence for agmatoploidy in this case. A further complication is added by the presence of hybrids which appear to occur quite frequently in some sections. As Stebbins (1971, p.20) points out, it is possible to obtain almost any chromosome number from a small series of basic numbers if an appropriate succession of crosses and formation of allopolyploids occurs. As a result of the peculiar cytology of this group, it therefore seems likely that in the older sections with complicated histories, it may be almost impossible to work out a reasonably accurate base number and karyotype evolution, because originally simple polyploid series may have been almost completely obscured by later aneuploidy and hybridisation. It is possible that identification of specific chromosome bands, using modern techniques of Giemsa staining (e.g. see Filion, 1974) or fluorescence staining might yield additional evidence and make it possible to trace the history of individual fragments, but the small size of most Carex chromosomes makes any analysis of this type difficult. Giemsa staining techniques have been used successfully on insect chromosomes with diffuse centromeres by Maudlin (1974) but I have not found any examples of their use in plant groups which do not have the normal localised centromere.

It is obvious from the above discussion that cytological studies in Carex are subject to rather more uncertainties than in most other Angiosperm groups. The large numbers of very small chromosomes present in many species make it very difficult to be sure of the counts obtained, especially for somatic cells, and it is often very difficult in some cases to distinguish multivalents and univalents at meiosis. In spite of these disadvantages, Davies (1956) and Faulkner (1970, 1972) were able to obtain a considerable amount of useful information from cytological studies on sections Flavae and Acutae, and it was therefore considered that such an approach would be of great value in the present study. A cytological survey was therefore undertaken with the following aims in mind:

1) To make chromosome counts for those species in which the chromosome number was unknown.
2) To confirm counts already made for the other species of the Vesicariae.
3) To observe chromosome pairing behaviour at meiosis in hybrids, both natural and artificial, to give some indication of the relationships between the species involved.

6.2 Materials and Methods

Chromosome counts were normally made on meiotic preparations. It was found that in root tips of C. rostrata, using the HCl and pectinase technique described by Faulkner, the chromosomes were too small and sticky to allow clear counts and it was almost impossible to obtain counts consistent to within one or two chromosomes. Both Davies (op.cit.) and Faulkner (1970) found some difficulty in obtaining good root-tip counts, and because of the difficulties involved, I did not use this technique extensively. Löve et al.(op. cit.) found that root tip counts were quite adequate and it is possible that sections vary in the ease with which these preparations can be made. In view of the difficulties involved in obtaining root tip counts, it would seem that these should be treated with some caution and that meiotic counts are more reliable. For the meiotic preparations made, young male spikes which were just emerging from the surrounding leaf sheaths and had not yet become coloured, were fixed in Carnoy's fixative containing enough ferric chloride to make the solution straw-coloured. After two weeks storage at room temperature, several anthers were dissected out and placed in a drop of acetocarmine on a slide, slightly squashed and the larger pieces of anther wall removed. It was found that preparations made very soon after fixing were not well stained and the two weeks storage period was necessary to provide good staining. The anther contents were then macerated with a brass rod, covered with a cover slip, heated gently, and then squashed by pressure on the cover slip. The preparations were ringed with rubber solution to prevent them from drying out too quickly. Photographs were taken on a Vickers Patholux microscope on Ilford Pan - F film, developed in Acutol.

The plants used were normally cultivated in the greenhouse, and since a number of them were subsequently utilised in crossing experiments, voucher specimens of these could not be retained. In the case of <u>C.rostrata</u>, counts were made on material collected in the

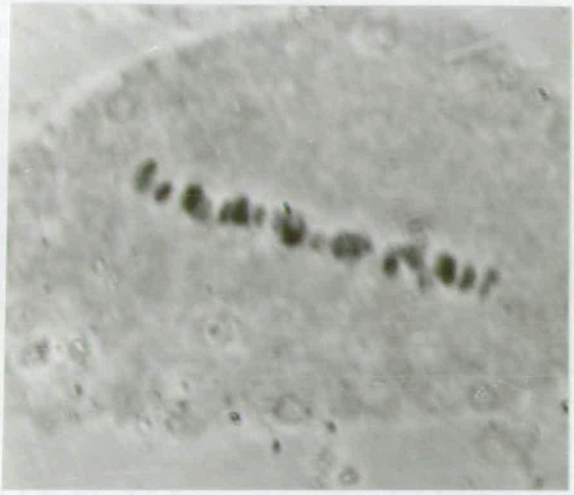
Fig. 6.1 Pollen development in <u>C.vesicaria</u> (x 2000)

a) Metaphase I - side view

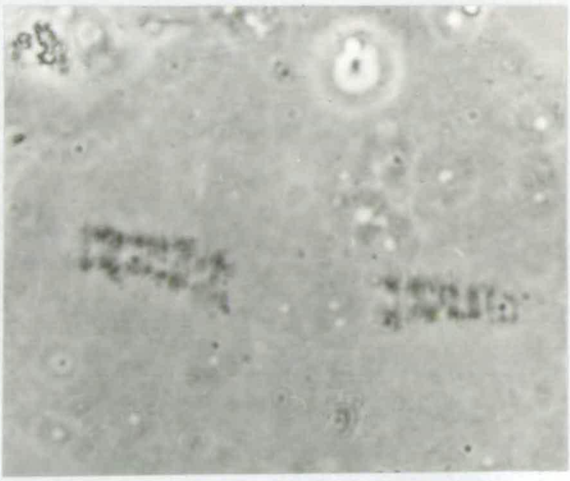
b) Anaphase II - side view

c) Prophase of pollen mitosis, showing pollen nucleus (large) and three smaller nuclei which will abort.

d) Telophase of pollen mitosis. The three small nuclei have not divided and are beginning to break down.



a



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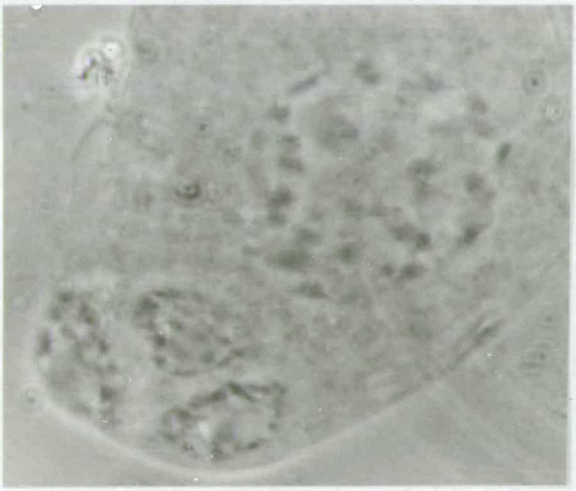


c

d

wild at Threipmuir reservoir and represented material of at least two different clones. It was found that the oldest flowers in the spike were at the middle and those towards the ends were younger. It was therefore usually possible to find at least one flower in the metaphase I stage in the spikes collected, and a range of pollen development stages could be observed. Metaphase I was found to be the most convenient stage, although counts could be made at pollen grain mitosis. Some of the stages of pollen grain development in C.vesicaria are shown in fig. 6.1.

6.3 Cytological observations

Counts were made for all of the British species of Sect. Vesicariae and a few species in related sections but because of difficulties in obtaining flowering material, species such as C.lasiocarpa were not investigated and the survey was therefore rather less extensive than had originally been intended. In addition, the hybrids produced from the biosystematic part of this study (with one exception) had not flowered by the end of the experimental work and these have not been investigated. It is intended that these shall be grown on for study at some later date.

6.3.1 Section Vesicariae

The results of chromosome counts made for members of this section are summarised in Table 6.1.

1) C. rostrata. Counts for this species were made on wild material collected at Threipmuir Reservoir, Balerno, Midlothian. The material was collected from two areas which on morphological grounds (cf. Ch. 8), were thought to contain two separate clones. All the material gave n = 37 or 38, although in a number of preparations, the chromosomes were somewhat sticky and it was difficult to be sure that

separate bivalents were being observed. It seems most probable that n = 38 is the correct number, since in each of the plates where n = 37 was recorded, a large chromosome association was present which was almost certainly a pair of bivalents stuck together. This is also the most frequently reported number in the literature. There was no obvious sign of any meiotic abnormality.

2) C.vesicaria. Counts were made on cultivated plants originally collected at Loch Tay (Perthshire) and Loch Ken (Kirkcudbrightshire). Both gave n = 41, a figure which agrees with most previous counts on European material. No abnormalities were observed (fig. 6.2).

3) C.saxatilis. Counts were made on five cultivated plants originally collected on Meall nan Tarmachan, Perthshire. All gave n = 40 and appeared to have regular meiosis with even-sized bivalents. These counts agree with most of the previously published figures for this species.

4) C.grahamii. Counts were made on material from the Meall nan Tarmachan clone only, using cultivated plants. This count is of some interest since there appears to be no previous record of a count for this species, either as C.grahamii in Britain or as C.stenolepis from Scandinavia. The number recorded for most of the plates observed was n = 41. In nearly all the cells investigated, two large chromosome associations were present. These could be interpreted either as large bivalents or trivalents (fig. 6.2, marked with arrow). If the bodies are trivalents, then the chromosome complement for C.grahamii is 39 II + 2 III and 2n = 84, and if they are bivalents, then the complement is 41 II, i.e. 2n = 82. There are also some rather small bivalents and it is possible that one or more of these is

Table 6.1 Chromosome counts.

Species	2n	No. of counts
C. rostrata	78	4
C. vesicaria	82	2
C. saxatilis	80	5
C. grahamii	82or84	3+
C. pseudocyperus	66	1
C. hirta	112	1

+ all from the same clone

Fig.6.2 Chromosome complements of _Carex_ spp. in Sect._Vesicariae_.

(all shown at metaphase II) (x 2000)

a) _C.vesicaria_

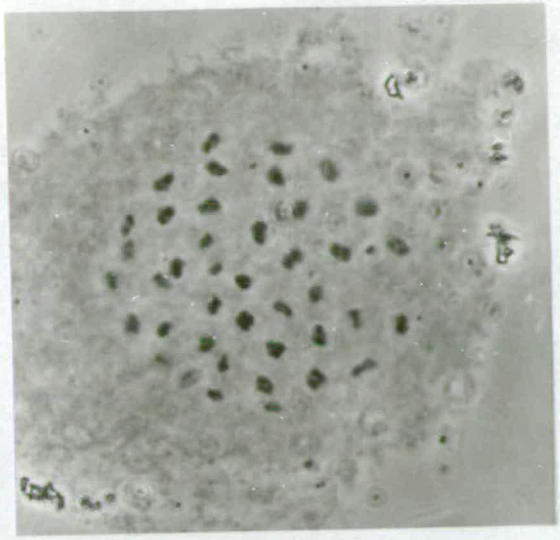
b)-c)_C.grahamii_ (from Meall nan Tarmachan).

d) _C.saxatilis_

e) _C.grahamii_ x _vesicaria_

Bivalent out of focus shown by dotted line.

Arrows show large bivalents or trivalents.



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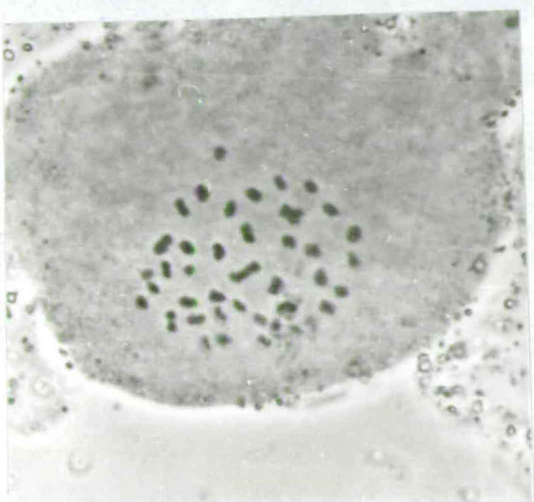

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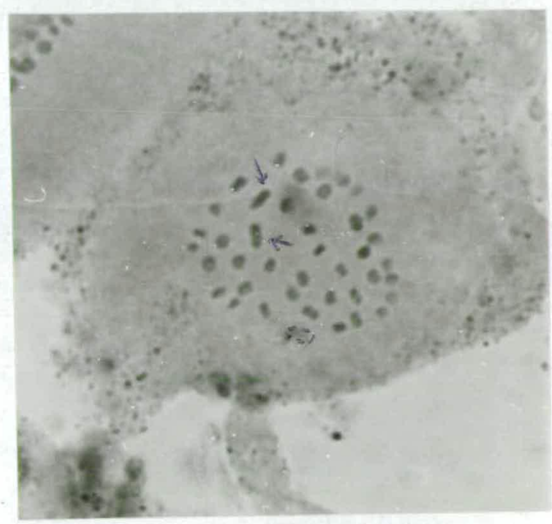

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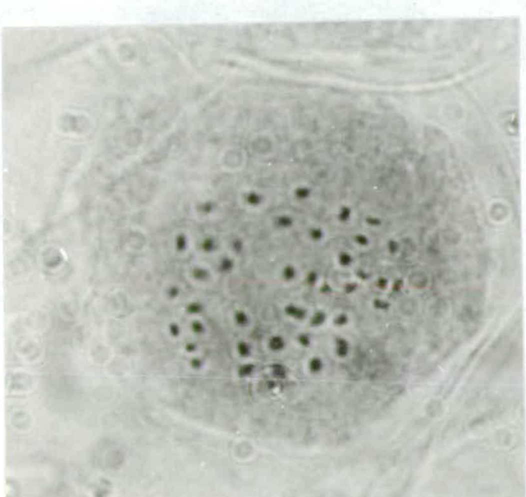

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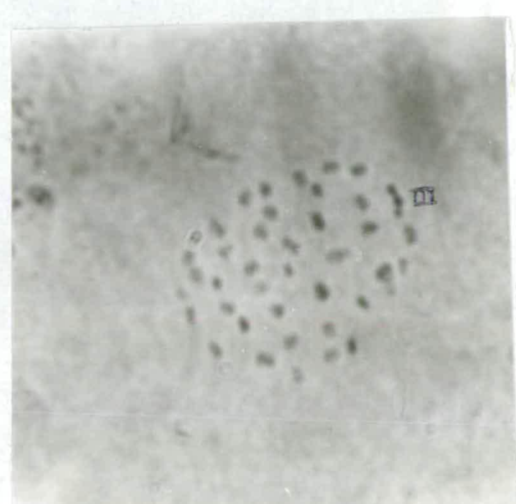


e

a univalent, but it is very difficult to distinguish univalents properly.

6.3.2 Related Sections.

Counts were made for two species from sections Hirtæ and Pseudocypereae. The numbers recorded were n = 56 for C.hirta and n = 33 for C.pseudocyperus. These are in agreement with published figures summarised in Fedorov (1969) and Moore (1973). No abnormalities were noted, but in C.pseudocyperus, in the later stages of pollen development at pollen grain mitosis, it was noticed that a large number of small nucleoli were present in the three aborting nuclei at the narrow end of the cell. There appeared to be as many as three nucleoli in each nucleus, in some of the cells at least.

6.3.3. Cytology of artificial hybrids

Only one hybrid plant had flowered by the end of the experimental work for this study. This was a C.grahamii x vesicaria cross, and flower buds were collected for cytological investigation. Chromosome pairing at Metaphase I was very regular, with 40 II and 1 III, giving a diploid number of 2n = 83 which is compatible with parental numbers of n = 42 and n = 41. The regular pairing would seem to indicate that there is a close genetic affinity between C.grahamii and C.vesicaria. No cells were found containing large multivalents of the type found in interspecific hybrids by Davies (1956). If C.grahamii is indeed a hybrid between C.saxatilis and C.vesicaria, it would appear that the latter species are very closely related, as far as cytological behaviour is concerned, because both the hybrid (C.grahamii) and its backcross with C.vesicaria have regular chromosome pairing with very little indication of any abnormality. Results from a direct cross between the two species are required to confirm this.

6.4 The Cytology of C.grahamii

In view of the widely held opinion that C.grahamii is of hybrid origin, (i.e. C.saxatilis x vesicaria), it was expected that some degree of meiotic disturbance would be present, since both Davies (1956) and Faulkner (1972) found extensive disruption of meiotic pairing and multivalent formation in their hybrid plants. In fact, there appears to be very little sign of meiotic abnormality and the majority of bivalents formed are quite normal. The only unusual feature observed was the presence of two bodies which are markedly larger than the other bivalents present. I have been unable to decide whether these are simply unusually large bivalents or trivalents, but the latter seems more likely since the bodies in question closely resemble the chain trivalents found by Faulkner (1972), although they are not sufficiently well resolved to confirm this. In addition, neither of the two proposed parental species possess any large bivalents which might be expected to appear in the hybrid. If these large bodies are trivalents, then the chromosome number for C.grahamii is 2n = 84 and if not, then 2n = 82. If the plant is in fact a hybrid between C.saxatilis (2n = 80) and C.vesicaria (2n = 82), then a number of 2n = 81 would be expected, with a maximum number of 40 bivalents and one univalent, and if this is the correct interpretation of the origin of C.grahamii, then the plants represented by the Meall nan Tarmachan clone must have undergone some cytological changes since the original hybridisation. The minimal change required to produce a number of 2n = 84 would seem to be (1) splitting of the univalent and association of the resulting fragments with two of the bivalents to form trivalents and (2) splitting of a bivalent to give a total complement of 39 II + 2 III. If the correct number is 2n = 82, then splitting of the univalent and association of the fragments to form a bivalent, or loss of the univalent and splitting of one of the remaining bivalents to give 41 II would seem to be the minimal change required. Another explanation for the anomalies in number might be that the original parental individuals were cytologically abnormal, but this seems unlikely since the existing material examined of both C.saxatilis and C.vesicaria in the Loch Tay area was cytologically unremarkable. It would also be possible to explain the Tarmachan C.grahamii clone as a peculiar form of C.vesicaria (with 2n = 82) but the presence of two large bivalents not found in other forms of the species still has to be explained. An alternative to this, of course, would be that C.grahamii is a totally independant species which has evolved separately and is not directly derived from C.vesicaria or C.saxatilis. This explanation is to a certain extent supported by the regular pairing behaviour of the chromosomes in this plant which in many ways resembles that of an established species rather than a hybrid. A fifth explanation might be that another parental species was involved, replacing either C.saxatilis or C.vesicaria, but it is very difficult to find any other likely species which combines an appropriate chromosome number and morphology, and seems to be closely enough related to make the cross likely.

If C.grahamii is to be regarded as a hybrid between C.saxatilis and C.vesicaria, two main conclusions may be drawn: - (1) some degree of chromosomal rearrangement has occurred, either immediately before the original hybridisation or at some time in the subsequent evolution of this taxon, (2) the high degree of regular pairing observed suggests that C.vesicaria and C.saxatilis are closely related. However, the cytological evidence does not permit any clear and unambiguous decision on the origins of C.grahamii.

6.5 Cytology and delimitation of Sections

The chromosome numbers reported for members of the Vesicariae and related sections are listed in Table 6.2. These are mainly derived from Fedorov (1969) and Moore(1973) and my own counts have also been included. The delimitation of the sections and the species included follows Kükenthal (op.cit), but where segregates not recognised by this author have been counted, these are included as separate species. Kükenthal's three subsections of Sect.Physocarpae have been separated to emphasize the differences which occur between them and in part this follows modern opinion, at least in separating the Lupulinae as a distinct section. A number of interesting points emerge from this table. With the exception of three counts of 2n = 60 for C.rostrata and C.physocarpa, and one of 2n = 40 for C.miliaris, the members of Sect. Vesicariae have consistently high counts of 2n = 74-86. In C.rostrata, there is only one count of 2n = 60 and there are 8 counts of 2n = 72 or higher, suggesting that this count (from Sweden) is not typical of the species as a whole, if it is not an error. The counts made by Wahl (1940) were from two plants of C.rostrata var. utriculata, both of which gave 2n = 82 and it therefore seems likely that this variety differs from the type in its chromosome number. The count for C.miliaris is atypical of the group as a whole and may be of considerable importance in its phylogenetic implications but more counts are required before any firm conclusions can be drawn. Unfortunately the source (Löve, 1954) gives no details of the locality or how many plants were examined and whether this was a meiotic or root tip count.

In contrast to the Vesicariae, the other subsections of Kükenthal's Physocarpae have lower chromosome numbers. The Tentaculatae

Table 6.2 - Chromosome numbers in <u>Carex</u> sect. <u>Vesicariae</u> and related sections.

Section	Species	Number	No. of counts made		Provenance
Vesicariae	miliaris	40	1		N. America
	rostrata	60	1		Scandinavia
		72-74	1		U.S.S.R.
		76	5	*	Europe
		82	2		U.S.A.
	laevirostris	74	1		Japan
		80	1		Japan
		82	1		Scandinavia
	vesicaria	74	2		Japan, N.America
		82	4	*	Europe
		86	1		Europe
	saxatilis	80	12	*	Europe, U.S.S.R. N. America, Greenland
		82	1		Scandinavia
	physocarpa	60	2		N. America
		c78	1		N. America
		c80	1		N. America
	membranacea	74	1		U.S.S.R.
		76-80	1		N. America
	grahamii	82 or 84	1	*	Scotland
Tentaculatae	dickinsii	64	1		Japan
	retrorsa	70	2		N. America
	tuckermanii	76	1		N. America
	lurida	64	1		N. America
		66	2		N. America
	baileyi	68	1		N. America
	squarrosa	56	1		N. America

Table 6.2 (cont.)

Section	Species	Number	No. of counts made	Provenance
Lupulinae	michauxiana	64	1	Japan
	folliculata	56	2	N. America
	intumescens	48	1	N. America
	grayii	52	3	N. America
		54	2	N. America
	idzuroei	58	1	Japan
	lupulina	56	2	N. America
		60	1	N. America
	lupuliformis	60	1	N. America
Pseudocypereae	pseudocyperus	66	5 *	Europe, N.America
	comosa	64	1	N. America
	capricornis	70	1?	Japan
	hystricina	58	1	N. America
	schweinitzii	60	1	N. America
Hirtae	lasiocarpa	56	2	Europe, N.America
	lanuginosa	78	1	N. America
	fedia var.miyabei	90	1?	Japan
	hirta	112	7 *	Europe, N.America
	trichocarpa	110	1	N. America
Paludosae	trifida	60	1	Macquarie Islands
	acutiformis	c38	1	Europe
		78	2	Europe
	riparia	72	3	Europe
	lacustris	74	2	N. America
	scabrifolia	82	1	Japan
	pumila	82	1?	Japan

* Counts made in the present study included.

range from 2n = 56 to 2n = 76, while the Lupulinae range from 2n = 48 to 2n = 64. Turning to the related sections, it is obvious that the Psuedocypereae have a small range of numbers which again are lower than those in the Vesicariae. In this case the range is from 2n = 58 to 2n = 70. The Paludosae have rather higher numbers, comparable to those of the Vesicariae, varying from 2n = 60 to 2n = 82, with the exception of one count of 2n = 38 for C.acutiformis. This was made by Reese (1951) on root tip material and does not appear to be typical of the species as a whole since there are two other counts of 2n = 78. It is possible that this was an aberrant individual or that some error in identification or counting was made. The Hirtae present a marked contrast to the other sections described above. The numbers in this section are very variable and range from 2n = 56 to 2n = 112 - a much wider range than in any of the other sections investigated and probably the biggest range reported for any one section.

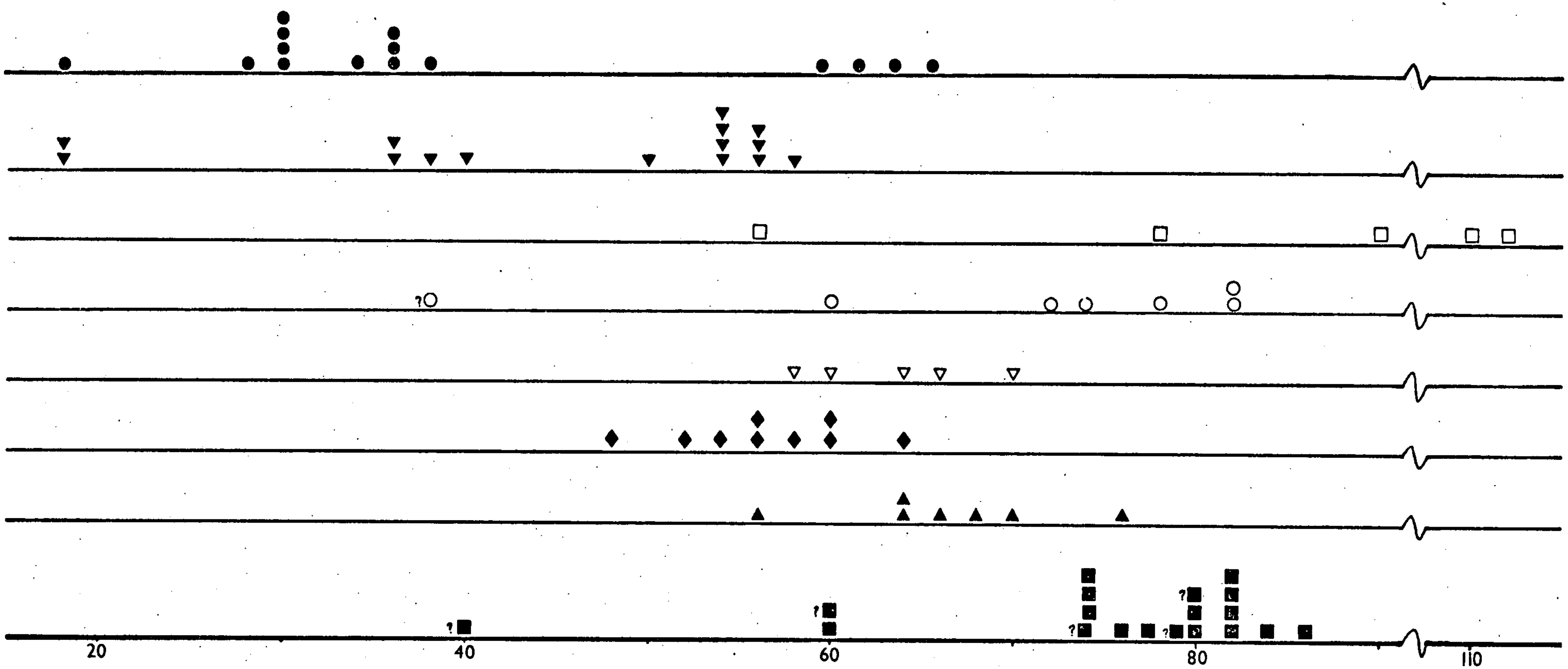
It would therefore seem from the evidence of the reported chromosome numbers, that there is a tendency for different sections to have comparatively narrow ranges of numbers which are often characteristic of the sections concerned. If this is the case, it would appear to lend some support for the separation of the Pseudocypereae from the Vesicariae and also for separation of the Lupulinae and Tentaculatae from the latter section. The Lupulinae also tend to have rather lower numbers than Sections Pseudocypereae and Tentaculatae and if Koyama (1962) is correct in placing C.michauxiana in the Pseudocypereae, this difference in range would be accentuated. The Paludosae on this evidence do not appear to differ significantly from the Vesicariae, having numbers which are comparable with the latter section. The tendency for related species to have similar numbers was noted by

Fig.6.3 Distribution of chromosome numbers in the *Vesicariae* complex and in Sects. *Montanae* and *Capillares*.

data from Fedorov (1969), Moore (1973), Reznicek & Ball (1974) & Koyama (1962).

Each chromosome number reported for each species is represented by a symbol (e.g. both 2n = 76 and 2n = 82 are given for *C. rostrata*). Dubious counts are indicated by a question mark (cf. text for details).

(■) *Vesicariae*	(▽) *Pseudocypereae*
(▲) *Tentaculatae*	(○) *Paludosae*
(◆) *Lupulinae*	(□) *Hirtae*
(▼) *Capillares*	(●) *Montanae*



?
?
?
?
?
?
20
40
60
80
110

Wahl (1940), and from the results given by Faulkner (1972) for Sect. Acutae, it is apparent that in this section, there is also a comparatively limited range of numbers, mainly between 2n = 66 and 2n = 86, although there are a few species e.g. C.bicolor, for which counts outside this range have been recorded. The British species at least of Sects. Spirostachyae and Flavae also seem to possess rather similar numbers, a fact which is of some interest since the data for natural hybrids shown in Fig. 2.3 suggests that these two sections are very closely related. The number 2n = 72 recorded for C.laevigata may reflect the increasing evidence that this species is closely related to members of this complex (cf. David, 1974).

Some sections, however, do not display a trend to restricted chromosome numbers. Of these, the members of Sect. Hirtae are the outstanding example, showing a very wide range of numbers within the same section. It could be argued that Sect. Hirtae is a heterogeneous group, containing species with both high and low numbers derived from different ancestral species. This view is supported by some authors, e.g. Kreczetowicz places C.hirta and C.lasiocarpa in separate sections. There are certainly differences in leaf and utricle anatomy (cf. Ch.5) and general morphology which would suggest that they are not especially closely related. This argument is weakened, however, when the counts reported for C.lasiocarpa, C.lanuginosa and C.fedia are considered. These were all considered by Kükenthal to be subspecies of C.lasiocarpa, but the numbers reported are 2n = 56, 78 and 90. It seems rather unlikely that species which are morphologically so closely similar should be derived from radically different sources and it is much more probable that some form of polyploidy is responsible for the diversity in chromosome numbers in this group.

The species of Sect. Montanae, which are probably not very closely related to any of the groups discussed above, also show a rather wide range of chromosome numbers, at least in the British species where the number varies from 2n = 18 to 68, but if Wahl's (1940) investigations of N. American species are included, there is a strong grouping of species around 2n = 30 to 36 and the extremes described above are not typical of the group as a whole. However, polyploidy must have occurred to some extent to produce the wide differences observed. Löve et al. (1957) found a series of polyploids in their investigation of Sect. Capillares, with diploids (2n = 18), tetraploids (2n = 36) and hexaploids (2n = 54) together with some aneuploids (2n = 38, 40, 56, 58). In this case, as can be seen from fig. 6.3, the counts for the species in this section fall into three well-marked groups at the successive polyploid levels, making interpretation of karyotype history relatively easy.

In spite of the variation shown by Sections Hirtae, Montanae and Capillares, it would appear that many of the large sections of Carex have surprisingly narrow ranges of chromosome numbers, in view of the diversity of the genus as a whole. It is not immediately obvious why such a situation should occur, since it is reasonable to believe that closely related species might be derived by polyploidy within a section, leading to a greater diversity in numbers as shown by Section Capillares. Two explanations seem possible - either the sections as delimited at present are unnatural, and are assemblages of forms at similar ploidy levels rather than related species, or the reported chromosome numbers reflect in some way the evolutionary history of the sections. It seems possible that a major evolutionary development leading to the formation of a new section might have been accompanied by a cytological discontinuity and that subsequent speciation was achieved with only comparatively minor changes in chromosome number and arrangement in most species. Occasional cases of further polyploidy might also have occurred. This type of explanation could well be applied to Sect.Montanae where one may envisage a preliniary series of polyploids with 2n = 10, 20, 30, 40 followed by extensive development at the 2n = 30 and 40 levels to give a complex of species at slightly above or below these levels forming the main bulk of the section, with occasional polyploids at a higher level, e.g. C.caryophyllea (2n = 62 to 68) and a few survivors at lower ploidy levels, e.g. C. pilulifera (2n = 18). It is possible that C.miliaris may represent a lower polyploid level in the Vesicariae but the number of 2n = 40 for this species requires to be confirmed before any more definite conclusions can be drawn. Together with the reports of 2n = 60 for C.physocarpa and other species, this count would suggest that a basic 2n = 40,60,80, polyploid series has occurred in the Vesicariae.

The use of chromosome numbers to delimit sections must obviously be treated with a considerable degree of caution. In most of those discussed, a large proportion of the species are cytologically unknown. This is especially true of the very interesting Southern hemisphere species in S.America, S.Africa, Australia and New Zealand. It seems highly probable that examination of these species will considerably alter the present views on karyotype evolution in the Vesicariae 'complex', although Sect.Vesicariae itself has no Southern hemisphere members. In the Hirtae, only three out of Kukenthal's 16 species have been investigated but the proportion is rather higher in most of the other sections. There is also a great lack of information on the species found in one of the richest areas for members of this group - the region stretching from the Himalayas to Japan where numerous species of Sects. Paludosae and Pseudocypereae occur.

Table 6.1 is shown in diagrammatic form in fig. 6.3, together with data for Sections Montanae and Capillares. From this and the evidence discussed above, a number of tentative conclusions can be drawn on the delimitation of sections in the Vesicariae 'complex', dividing them into four main groups:-

(1) Sections Vesicariae and Paludosae with few exceptions have high chromosome numbers which distinguish them from the rest of the complex. It is not possible to separate these sections on the basis of chromosome number.

(2) Sections Tentaculatae and Pseudocypereae have rather lower numbers which are quite well distinguished from group 1 but overlap to some extent with the next group.

(3) Sect. Lupulinae with the lowest chromosome numbers in the complex. It is of interest that Sect. Tentaculatae, which tends to be intermediate between the Lupulinae and Vesicariae, should also be intermediate in chromosome numbers.

(4) Sect. Hirtae with a very wide range of numbers does not resemble any of the other sections. This is probably a heterogeneous group.

CHAPTER 7 BIOSYSTEMATICS

7.1 Introduction

This chapter summarises the series of crossing experiments carried out on members of Sect. Vesicariae and related sections. The experimental programme was undertaken with three main aims in mind:

(1) To clarify the relationships of the species within sect. Vesicariae by observing their crossability with each other, with special emphasis on C. grahamii.

(2) To produce artificial hybrids which could be compared with material of putative natural hybrids, with regard to both their morphology and cytology.

(3) To examine the degree of crossing possible between members of the Vesicariae and related sections such as Sect. Pseudocypereae, in order to determine whether crossability data would support the merging of these groups into one section as suggested by data on natural hybrids.

The main points at which crossability was estimated were (1) at seed production (% seed set) and (2) germination and growth of the hybrid seed. In a few cases, pollen germination and pollen tube growth were also examined. Unfortunately, because of limitations of lack of flowering material and time, it has not been possible to make the survey as complete as had originally been intended. In particular, the series of intersectional crosses which were completed was very limited, and is not sufficient to answer in detail the questions posed above, but some interesting results have been obtained which are at least suggestive even though they do not provide conclusive answers. Since only one flowering hybrid plant has been obtained during the time available for this research, the fertility and cytology of the F_1 generation for the majority of crosses has not been investigated.

7.2 Materials and Methods

The plants used for crossing experiments were grown in the greenhouse and flowering occurred from early March to mid-May. In the case of C.saxatilis in particular, it was necessary to collect fresh plants for each experimental season since this species did not grow well under greenhouse conditions, although the plants survived long enough to set seed.

Inflorescences to be used as female parents were emasculated as soon as the spikes appeared above the leaves and were covered in envelopes similar to those used by Alexander (1975), made of two layers of lens tissue. These were found to be very light, and when closed off at the bottom by two paper clips, could be left on the inflorescence without any support. The only disadvantage of these envelopes was that if they were wetted, the enclosed spikes were sometimes attacked by moulds and the increased weight occasionally broke the culm. In a few cases, the stigmas had already begun to emerge when the female spikes appeared. These were used only if there seemed to be no danger of contamination from nearby male spikes. It is possible that some slight contamination occurred in some of the crosses since out of several inflorescences which were emasculated and covered, two produced one or two seeds per inflorescence, but this problem did not appear to be serious. In C.saxatilis, all of the material collected for one season (1973) produced only male spikes and inflorescences with aborted female spikes. As a result, this species could not be used as a female parent during that season.

Pollen was collected in small plastic vials and then stored in a refrigerator at 4^{o}C. It was found that pollen stored in this way remained viable for more than a month after collection, when tested on fresh stigmas, but most pollinations were carried out before the pollen was more than one or at most, two weeks old. Pollen was dusted on the stigmas as evenly as possible, using a small brush which was washed in water containing detergent and rinsed in alcohol between different pollen sources. In most cases a large number of pollen grains were deposited for each ovule. In C.saxatilis and C.pseudocyperus pollen production was rather limited and in crosses where these species were the male parents, the pollen density was probably lower than in most of the others. The female spikes were pollinated three to four days after the emergence of the stigmas which were then more or less fully expanded though the utricles at this stage were normally not inflated. C.vesicaria was usually found to be protogynous, but in C.rostrata, both protogynous and protandrous plants were found. I saw no examples of the aberrant flower and inflorescence types noted by Faulkner (1970), although these were found in plants of C.sylvatica and C.pulicaris which were growing in the same greenhouse.

Pollen germination was checked in a few cases by staining pollinated stigmas with cotton blue in lactophenol. This produced an intense blue colour in the exposed parts of the pollen tubes but did not stain the tubes which had penetrated into the style. The cotton blue staining method described by Murty (1971) was also tested but did not give satisfactory results. Although it was not possible to calculate the exact pollen percentage germination, it was usually possible to obtain an approximate figure by counting the number of visible pollen tubes. It was found that individual flowers which had been removed from an inflorescence, could be kept fresh for at least two or three days at room temperature by inserting them in small slits in a thin layer of 1% water agar at the bottom of a petri dish. Pollen appeared to germinate normally on the stigmas of flowers treated in this way. If the dishes were kept in a refrigerator at 4°C., the stigmas stayed fresh for at least 6 to 7 days. Although this system was not used extensively in the present study, it may be of some value in an investigation of incompatibility and pollen germination since about 100-200 flowers can be obtained from a single spike in the larger species and these may be tested individually with pollen of any desired type, so that a large number of tests can be carried out on a single plant. It is not known if fertilisation can occur in separated flowers, but long well-formed pollen tubes are produced. A similar system was used by Lundquist (1961) in his investigations of incompatibility in grasses,but in this case, sucrose and boric acid were added to the agar, and only the gynoecium of each flower was used. This system does not appear to have been widely used. Martin's aniline blue fluorescence staining method (Martin, 1959) was found to work very well, on _C.vesicaria_ at least, and pollen tubes could be followed down the style as far as the ovule. Since pollen cell wall proteins appear to play a significant role in incompatibility systems, the commassie blue protein stain suggested by Howlett, _et al._ (1973) was tested on _C.vesicaria_ pollen with _Bellis perennis_ as a control since this method had been developed mainly in species of Compositae. It was found that some protein material was given off by the _Carex_ pollen grains but this appeared to be much less abundant than in the _Bellis_ pollen (fig. 7.1).

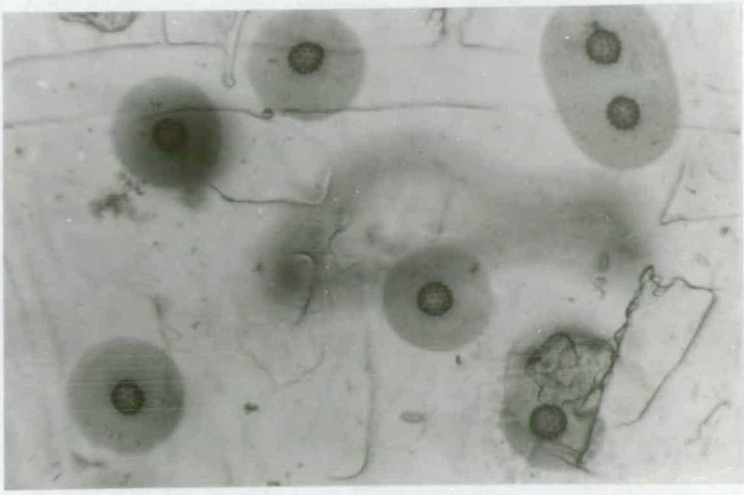
Ripe fruits of possible hybrid origin were collected in paper envelopes and stored at room temperature for about a month and samples were then planted out on 1% water agar in a fluctuating

Fig.7.1 Production of protein by pollen grains.

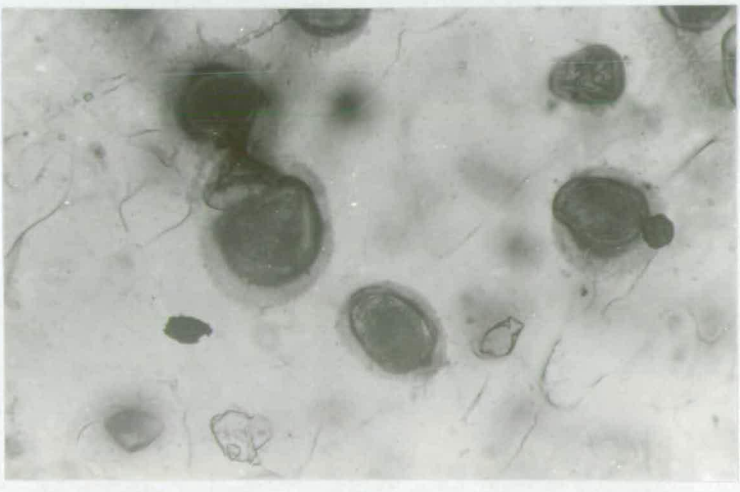
a) *Bellis perennis*

b) *Carex vesicaria*

Pollen grains stuck to double-sided sellotape and flooded with the coomassie blue stain described by Howlett *et al.* (1973). (x 225)



a



b

temperature environment as described in Ch.2. In most cases, germination occurred readily and the seedlings produced were healthy and vigorous. With one possible exception (c.f. sect.7.4), there were no obvious indications of inviable hybrid plants, although occasional seedlings grew slowly or appeared to be very weak.

7.3 Intrasectional Crosses

In this group, two main sets of crosses were carried out -

(1) intraspecific crosses to test the seed set in normal pollination within the species and to investigate the possibility that an incompatibility system was present.

(2) interspecific crosses between the four British species in the Section *Vesicariae*.

7.3.1 Intraspecific crosses. Intraspecific crosses were made only for *C.rostrata* and *C.vesicaria* because of limitations of flowering material available. The results obtained are summarised in Table 7.1. Of the crosses listed there, nos.4,5,7 and 9 were self pollinations. It can be seen that seed sets for these did not exceed 12%, and were frequently lower, suggesting that some form of incompatibility system is operating in both species, preventing abundant seed set from self pollination. Crosses 1 to 3 were made using a set of six plants of *C.rostrata* from two widely separated areas at Threipmuir reservoir. It was assumed that plants 73-75, which came from one small area, were members of a single clone, while plants 76-78 which came from the second area were members of another quite separate clone. This seemed highly likely since the two areas were separated by a considerable distance and were on opposite sides of the reservoir. If this assumption was correct, then crosses 1 and 3 should have been self pollinations while cross 2 was a cross pollination. The results obtained would seem to support this interpretation, since the two

Table 7.1 Intraspecific crosses

Cross No.	Plants used[a]	% Seed set	Pollen age+ (days)	Pollen germination 1 hr.	24 hr.
1	rostrata 77 x rostrata 78	0	0	0	-
2	rostrata 76 x rostrata 74	45	0	52	-
3	rostrata 73 x rostrata 75	0	0	0	-
4	rostrata 295 x rostrata 295	9	4	-	-
5	rostrata 399 x rostrata 399	0	0	-	-
6	rostrata 405 x rostrata 399	5	0	-	-
7	vesicaria 95 x vesicaria 95	11	4	5	20
8	vesicaria 95 x vesicaria 96	5	4	5	5
9	vesicaria 96 x vesicaria 96	12	4	5	20
10	vesicaria 97 x vesicaria 96	10	4	35	35
11	vesicaria 96 x vesicaria 97	12	0	5	10
12	vesicaria 95 x vesicaria 97	19	13	-	-
13	vesicaria 97 x vesicaria 95	44	13	-	-
14	vesicaria 99 x vesicaria 394	15	1	-	-
15	vesicaria 396 x vesicaria 96	90	0	-	-

+ in this column, O indicates that pollen was collected on the same day that the cross was carried out.

- not tested

a in each case, the female parent is first.

self pollinations gave no seed set, while the cross produced 45% fertile seeds. It can also be seen that pollen germination figures in this case reflect the seed set figures with no germination in the 'self' pollinations and abundant germination in the 'cross' pollinations. A further series of crosses was carried out on a set of three plants of C.vesicaria which had been collected at Loch Ken (Kirkcudbrightshire). All of these came from a small area (c.50 x 10 m.) and were tested for seed set and pollen germination. The results in this case were more difficult to interpret. Crosses 7-11 would suggest that all three plants belong to the same clone or at least possess the same incompatibility alleles since the seed sets were all very low and the cross pollinations gave results which were very similar to those from the self pollinations. Pollen germination is also very low when estimated shortly after pollination (1 hr.), and although the percentage increases considerably after 24 hours, it is still rather low (5-20%) when compared with the cross pollination figure obtained in cross 2 for C.rostrata (50%). It is also much lower than in some interspecific crosses, since a figure of 78% germination was recorded for a C.grahamii x rostrata cross and even pollination of C.vesicaria by C.laevigata pollen gave 30-40% germination. In these crosses, the pollen germination rate does not correlate very well with seed set. Cross 10 showed higher pollen germination rates than the others but the seed set was not significantly higher. Difficulties in interpretation arise when crosses 12 and 13 are considered. Cross 12 produced 19 fertile seeds and although this is rather higher than the seed sets shown in the preceding crosses in this series, it is still quite low and could probably be explained by chance variation. Cross 13, however, gave a seed set approaching 50% and it is not easy to explain this result if all three plants belong to the same clone. It is possible that unilateral fertility of this type could occur if it is assumed (1) that two incompatibility loci are present, (2) that all three plants have different genetic compositions and (3) that one plant (97) is homozygous at one locus. This system is summarised in Fig.7.2. From this it can be seen that the only successful pollination which can occur is from plant 95 to plant 97 and all other combinations are incompatible. Lundquist et al. (1973) suggest that condition (3) may have occurred in some of the plants which they studied in their investigation of rather complex three locus systems and it is known to be common in sporophytic systems (Williams, 1964). A further assumption which must be made is that the alleles contained in the pollen grain must both be different from those in the style for successful pollination. In the grasses, a difference in only one locus is sufficient to allow pollination (Arasu, 1968). Other explanations may be possible if dominance effects are included and could possibly be worked out for a one locus system, but the data available give no means of distinguishing between different models. It is obvious from the above, however, that it is necessary to postulate a rather complex incompatibility system to explain the results of crosses 7-13, and it therefore seems much more likely that some environmental factor was responsible for the breakdown of the incompatibility reaction between plants of the same clone. One possible explanation may lie in the fact that both the stigmas and the pollen used in cross 13 were older than those used in previous crosses and this may have led to breakdown of some part of the incompatibility mechanism, allowing fertilisation to occur. This explanation is to a

Fig.7.2 Hypothetical allelic system to explain the results of intra-specific crosses 7-13.

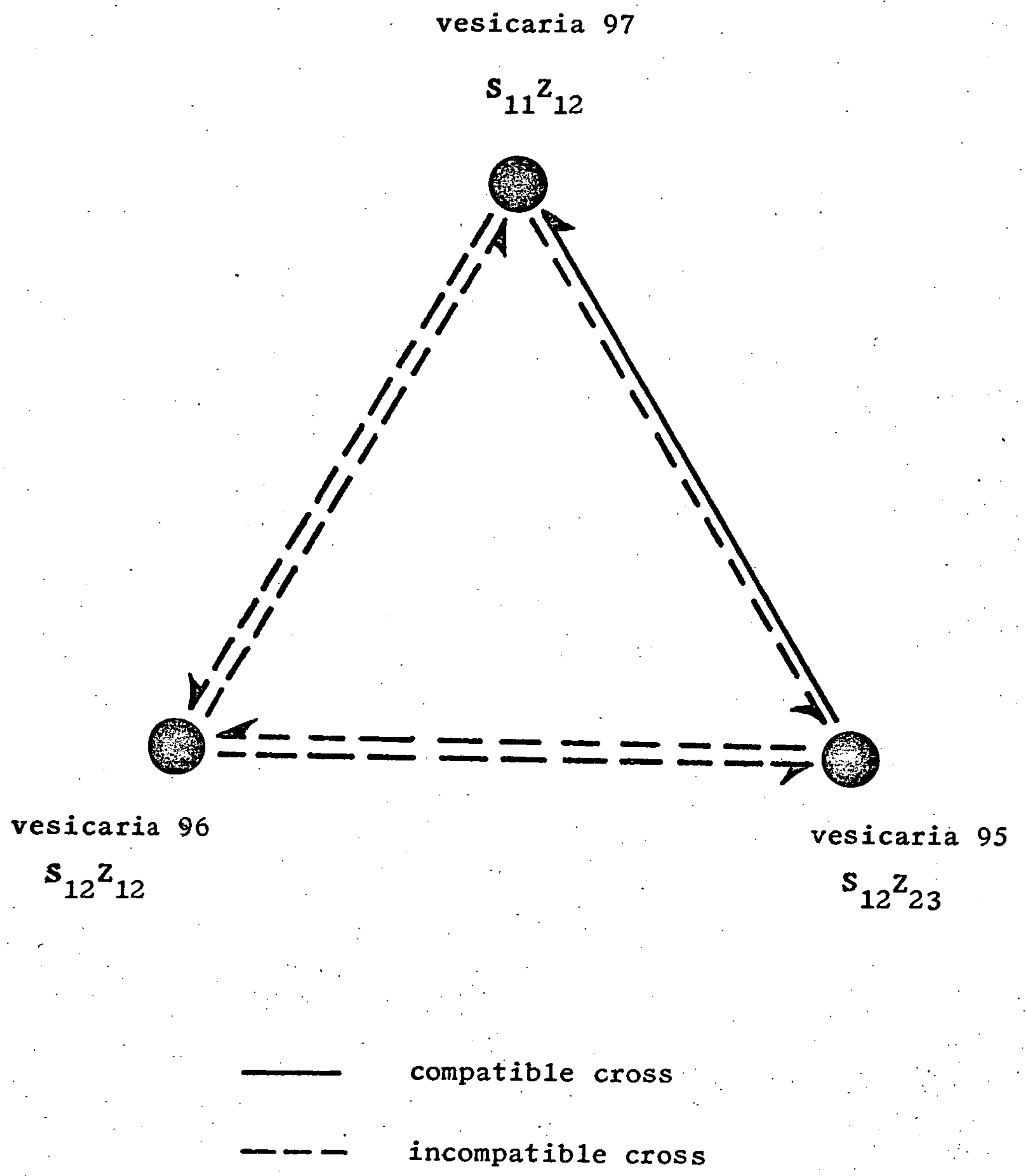


Fig.7.2 Possible incompatibility allele complements for <u>C.vesicaria</u> plants 95-95.

certain extent supported by the higher seed set in cross 12 where the pollen was also older, although the stigmas were of normal age. In cross 10, the stigmas were again a little older (5-6 days) than in the other crosses and there was an increase in pollen germination rate although this did not appear to be reflected by a higher seed set. It has been established that environmental factors such as temperature, season and plant age may strongly affect the efficiency of the incompatibility reaction in many plants (cf. Townsend, 1971). It therefore seems highly probable that an increase in seed set will result when older stigmas and pollen are used.

In the above discussion, however, the results of the 'cross' pollinations must be borne in mind. Out of four 'cross' pollinations, two gave 50% seed set or more, one gave 15% and one gave 5%. These results are surprising since most of these pollinations would be expected to give high seed sets. Faulkner (1970) obtained seed sets ranging from 40 to 100% in his intraspecific crosses. It could be argued that in those crosses giving low fertility, the plants possessed the same incompatibility alleles, but this seems unlikely since the plants in both cases came from widely separated localities and the probability of finding two plants of the same genotype under such conditions is very low. It would therefore seem likely that the seed set produced in intraspecific crosses between plants of different genotypes may vary widely, depending on environmental factors, and will not necessarily show high fertility.

In the self pollinations examined, some degree of disturbance of pollen germination occurred and this seemed to be rather more common in <u>C.rostrata</u>. The malformation of pollen tubes noted by Faulkner (1970) was not especially obvious, and in <u>C.vesicaria</u> many apparently normal pollen tubes could be seen in some of the self-pollinated stigmas. This was best seen when stigmas were examined 24 hours after pollination and it is possible that slow growth of 'self' pollen may occur. It also implies that a barrier to fertilisation may also be present in the tissues of the style, inhibiting the growth of incompatible grains.

7.3.2. Interspecific crosses

The results of the interspecific crosses made within sect. Vesicariae are summarised in Table 7.2. As far as possible, all the possible combinations of crosses and reciprocals of the British species were tested at least once. In some cases, very few replicates of the crosses have been made because of shortages of material. Two major features emerge from a preliminary study of these figures - (1) the seed sets produced are not consistent when the cross is repeated, e.g. the same species pair (C.vesicaria and C.saxatilis) in crosses 1, 2 and 3 gave seed sets of 0 to 50%. Even when the same individual plants were used, the results are not necessarily very similar (e.g. crosses 6 and 7). (2) reciprocal crosses rarely, if ever, give similar seed set levels, e.g. crosses 13 and 15. With the observed variation between replicates, it is perhaps not surprising that there should be a lack of agreement between reciprocal crosses, and in any case, this phenomenon is well-known from other studies, e.g. see the account of hybridisation experiments in Avery et al. (1954).

It is possible that in one, or perhaps two, of the crosses listed, some contamination has occurred, leading to self pollination either during the pollination or at some later stage. Cross 16 gave a very high seed set which seems rather surprising since this was a

Table 7.2 Interspecific crosses (within sect. Vesicariae)

Cross No.	Plants used[a]	% seed set
1	vesicaria 302 x saxatilis 311	47
2	vesicaria 395 x saxatilis 385	25
3	vesicaria 96 x saxatilis 388	0
4	saxatilis 384 x vesicaria 394	11
5	saxatilis 390 x vesicaria 394	0
6	vesicaria 394 x rostrata 404	17
7	vesicaria 394 x rostrata 404	38
8	rostrata 301 x vesicaria 99	15
9	vesicaria 395 x grahamii 392	29
10	grahamii 309 x vesicaria 99	65
11	grahamii 393 x vesicaria 394	0
12	rostrata 301 x saxatilis 311	25
13	rostrata 404 x saxatilis 385	0
14	rostrata 404 x saxatilis 388	30+
15	saxatilis 385 x rostrata 404	48
16	rostrata 301 x grahamii 310	90++
17	rostrata 301 x grahamii 310	65
18	rostrata 404 x grahamii 392	0+++
19	grahamii 310 x rostrata 300	20+
20	grahamii 148 x rostrata 406	59
21	saxatilis 130 x grahamii 214	7
22	saxatilis 384 x grahamii 392	35
23	grahamii 309 x saxatilis 312	70
24	grahamii 392 x saxatilis 388	23

\+ some nutlets empty

++ possibly contaminated

+++ about 40% empty nutlets produced

a in each case, the female parent is first

Table 7.3 Average seed sets in Interspecific Crosses

Parents female male	rostrata	vesicaria	saxatilis	grahamii
rostrata	xx	30	50	40
vesicaria	15	xx	5	35
saxatilis	20	25	xx	45
grahamii	35	30	35	xx

cross between C.rostrata and C.grahamii. Subsequent examination of the plants grown from the seeds obtained showed that in their vegetative morphology and anatomy they were identical with the female parent, C.rostrata, and it therefore seems almost certain that this cross was contaminated with C.rostrata pollen. It has therefore been omitted from subsequent result tables. It is possible that contamination has also occurred in cross 17 between the same species pair since the pollen source was identical for both and a high seed set was also obtained.

The differences in seed set between replicates of the same cross make it very difficult to use this type of data as an indicator of crossability in Carex, and it is questionable whether or not such data may ever provide any reliable evidence of the genetic affinity between Carex species and the degree of crossing possible. Faulkner (1970) made considerable use of seed set data with the aid of statistical tests and transformations designed to produce a normal distribution in his data, but this required a large number of replicates to give an adequate statistical basis for the work. In the present study, the number of replicates made was not sufficient for any meaningful statistical analysis to be made and I have therefore summarised the results of the crosses as simple arithmetic means of the seed set. Although this is probably not valid in statistical terms, it provides a convenient method of summarising the data and probably distorts it no more than any of the other methods of analysis which might otherwise have been applied. The mean seed sets for different species combinations are shown in table 7.3, where crosses and reciprocals are included. From this it can be seen that:-

(1) All of the crossing combinations attempted between the British members of sect. Vesicariae give some production of fertile seeds.

(2) The average seed set obtained over a number of replicates was rarely higher than 50% and usually considerably less.

(3) The reciprocals frequently gave different seed sets, e.g. C.saxatilis x vesicaria and the reciprocal cross.

It is interesting that C.rostrata gave relatively high seed sets when crossed with C.saxatilis and C.grahamii, since it differs from the other species in the section in its anatomy and chromosome number, as well as in morphology. Crosses between C.saxatilis and C.vesicaria do not give very high seed sets but C.grahamii appeared to be highly interfertile with both of these species.

The later stages of the sterility barriers between the species are probably much more informative when relationships between species are considered. Unfortunately, it has not been possible to study these in any detail because of difficulties in obtaining flowering material of hybrids within the time available for this research. Plants have been grown from at least one cross between each species pair, except in the case of C.vesicaria and C.rostrata in which the hybrid seeds failed to germinate. The test was, however, made on a very small batch of seeds and requires to be repeated, both for this cross (No.8) and for later crosses between these species which could not be tested in the time available. In general, with the one exception noted above, it would appear that all the British members of sect.Vesicariae are capable of crossing to produce hybrid seeds and at least vegetative hybrid plants. Germination of seeds was usually quite good and growth of the young seedlings was comparable to that shown by the pure species. All the plants produced from crosses within sect.Vesicariae have shown quite vigorous vegetative growth and do not appear to be stunted or otherwise abnormal. The plants have been kept growing and it is intended that at least meiotic preparations and herbarium specimens of hybrid plants should be obtained for future studies. At the time of writing, only one hybrid - C.grahamii x vesicaria - has produced flower buds. An examination of meiosis and formation of pollen grains suggests that fertility would not be greatly reduced since chromosome pairing was fairly regular and a high proportion of apparently normal pollen was produced (cf. Ch.6).

7.4 Intersectional Crosses

The series of intersectional crosses carried out is summarised in Table 7.4. It had been hoped to carry out a more extensive crossing programme but this was not possible in the time available. In particular, the material of C.lasiocarpa and C.riparia which had been collected could not be induced to flower, and no crosses were possible using these species. Two points emerge from Table 7.4:

(1) crosses between Sect.Vesicariae and Sects.Hirtae and Pseudocypereae produced no fertile seeds (with the exception of cross 6 which is discussed below), suggesting that a strong barrier to hybridisation is present either at the stage of pollen tube growth or one of the later stages of fertilisation and early development of the embryo.

(2) crosses between Sects. Hirtae and Pseudocypereae produced moderately high seed sets, although some of the nutlets produced were empty and contained no viable embryo or endosperm.

Three plants were obtained from the C.hirta x pseudocyperus crosses 7 and 8 and these were found to be very slow growing. Although one of these began to form a culm and inflorescence, this died off before flowers could be produced. There were no obvious signs of disease in

Table 7.4 Intersectional crosses

Cross No.	Plants used[a]	% seed set
1	vesicaria 97 x hirta 106	0
2	vesicaria 97 x hirta 106	0
3	vesicaria 97 x hirta 106	0
4	vesicaria 96 x pseudocyperus 44	0
5	vesicaria 99 x pseudocyperus 44	0
6	pseudocyperus 44 x vesicaria 99	65+
7	hirta 106 x pseudocyperus 44	25
8	hirta 106 x pseudocyperus 44	45

a in each case, the female parent is first.

+ possibly contaminated

these plants, and it was assumed that some form of hybrid breakdown occurred. This explanation, however, requires to be confirmed by growth of further plants from seed collected from these crosses.

In the case of cross 6, the seed set was very high, in sharp contrast to the reciprocal cross which produced no seeds. Plants grown from cross 6 seeds were found to be identical with the female parent in their vegetative morphology and anatomy and it is therefore assumed that contamination with pollen of C.pseudocyperus occurred at some point, hence permitting self-pollination.

7.5 Discussion and conclusions

The evidence available from intraspecific crosses suggests that some form of self-incompatibility system is operating, at least in C.rostrata and C.vesicaria, and probably in the other members of Sect.Vesicariae. Faulkner (1972) found that members of Sect.Acutae were also self-incompatible and it therefore seems likely that this condition may be common in the genus as a whole or at least in subgenus Carex. Since pollen grains in the Cyperaceae are normally shed at the three-nucleate stage (Davis, 1966), and Faulkner (1972) found that pollen tubes in an incompatible cross were usually malformed and did not penetrate the stigma, it seems most probable that some form of sporophytic system is present in Carex (cf. Arasu, 1964). In many of the crosses examined in the Vesicariae, however, it was noted that a few apparently normal pollen tubes were formed, even in self pollinations, suggesting that the incompatibility reaction may also be effective in the style as is usually found in gametophytic systems. This was most obvious in C.vesicaria pollinations, especially if some time had elapsed between pollination and examination (c. 24 hrs.). In C.rostrata, there was usually very little pollen germination in self pollinations.

It is obvious that the blocking of self fertilisation is not completely effective under artificial pollination conditions since some fertile seeds were produced even in self pollinations. This phenomenon is well known in a number of predominantly self incompatible species and is often referred to as 'pseudo-compatibility' (Williams, 1964). The degree of pseudo-compatibility shown by C.vesicaria appears to be slightly greater than in C.rostrata if seed set figures for incompatible crosses are compared, but this needs to be investigated more closely. This is perhaps related to pollination biology in the two species since C.vesicaria often grows in more sheltered habitats where wind pollination is presumably less effective and a greater degree of self pollination may be maintained by selection, ensuring seed set of adequate proportions.

The presence of an incompatibility system in Carex spp. may have profound biological consequences which must be considered in relation to fertility in these species under field conditions. As an example, C.rostrata, which is very vigorous in its vegetative growth, may frequently occur in single clonal stands e.g. along the edges of a small pond. In this case, most of the pollen reaching the stigmas would be 'self' pollen, leading to very low fertility for the whole clone since the amount of 'cross' pollen from compatible clones is likely to be rather small in a local species such as C.rostrata. In many stands, the inflorescence is not raised much above the level of the upper leaves during anthesis and wind pollination is therefore probably less efficient than in those species where the inflorescences are borne some distance from the ground and freely exposed to the wind. It was found that in some seed samples collected from small colonies, the percentage of good seed was very low. One colony of

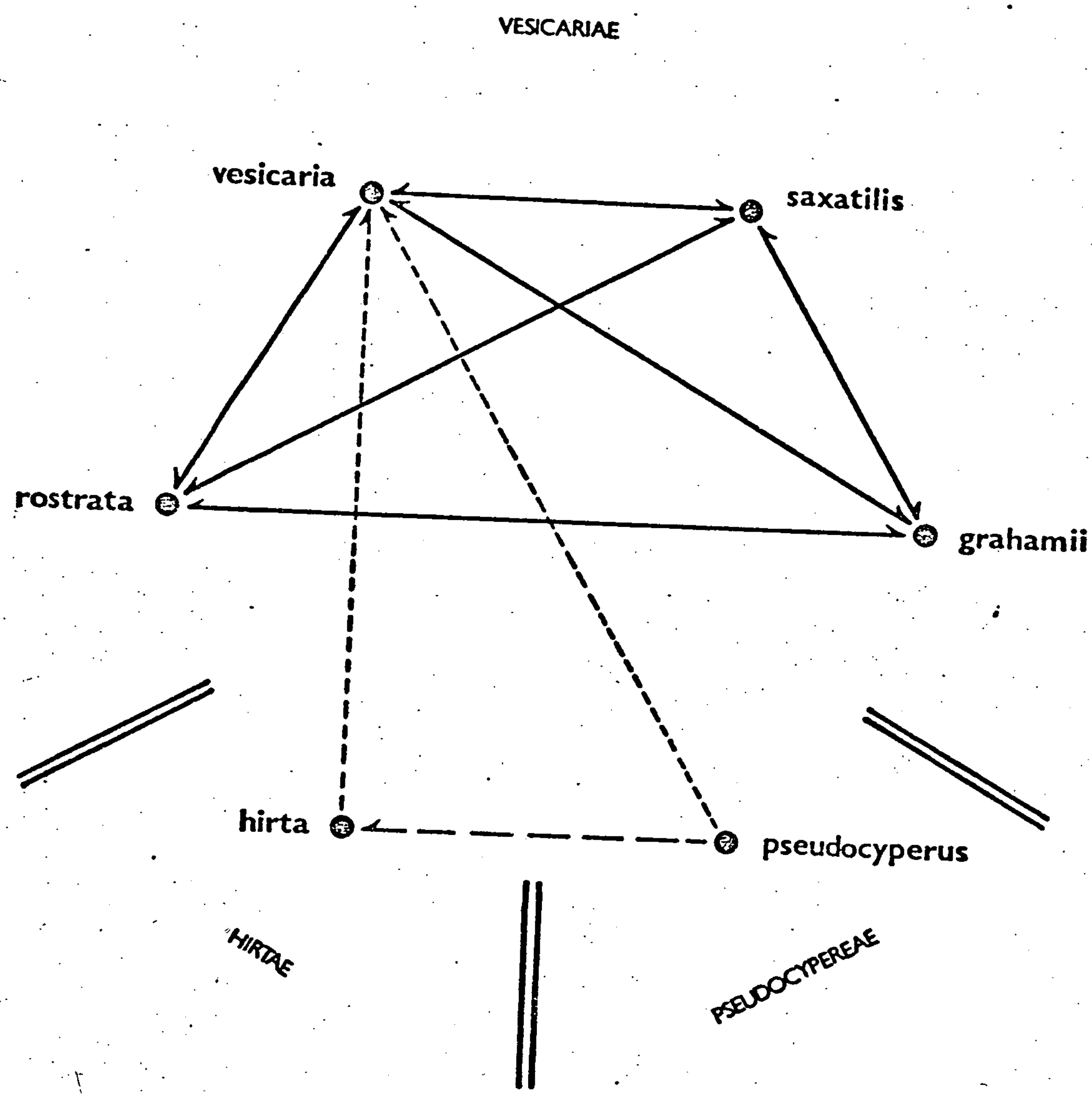
Fig.7.3 Summary of intra- and inter-sectional crossing relationships.

viable F_1 produced

inviable F_1 produced

no viable seeds formed

arrows show direction of pollen transfer


VESICARIAE
vesicaria
saxatilis
rostrata
grahamii
hirta
pseudocyperus
HIRTAE
PSEUDOCYPEREAE

C.rostrata at Tamdhu, Knockando, Moray, produced less than 5% fertile fruits, although the empty utricles were fully inflated and gave the appearance of high fertility. In sites such as Threipmuir, however, where a large number of clones occur within a relatively small area, fertilities are usually much higher and reached at least 25-30%. Very low seed sets have also been observed in C.flacca by Taylor (1956) who suggested that this may be genetically determined since it occurred at the same site over a number of years. The incompatibility system may thus be very important in the reproductive biology of at least some Carex species, leading to low fertility in many small, isolated stands and implying that individual clones must be relatively long lived and vegetatively vigorous if the species is to succeed.

With regard to the interspecific crosses, the results obtained (summarised in fig. 7.3) suggest that crosses within Sect. Vesicariae can occur quite readily, with production of viable F_1 plants, but it is not known at present whether all of these are capable of flowering and setting seed. The C.grahamii x vesicaria hybrid has so far produced flower buds only, but examination of the pollen grains suggests that it should be moderately fertile since very few deformed pollen grains were seen. The intermediate plants found in the Grantown-on-Spey population (c.f. Ch.8) were more or less completely sterile, but it is not clear at present whether this would occur in any of the other hybrids produced, or indeed whether it would be detectable under experimental conditions. Crosses with members of other sections appear to produce no viable seeds and suggest that a strong intersectional barrier exists, acting between the stages of pollination and embryo and endosperm formation. The crosses between sects. Hirtae and Pseudocypereae are of considerable interest because some seeds were produced although these sections are not normally regarded as being closely related. In this case, a crossability barrier seems to occur in the F_1 generation where the plants are rather weak and appear to be unable to flower successfully, but this requires to be confirmed by further tests. The presence of such barriers between sections is a strong argument for their retention in their present form since they seem to reflect basic discontinuities in the possible flow of genetic material between species. In biosystematic terms, Sects. Vesicariae and Acutae are probably equivalent to coenospecies which are incapable of exchanging genes with other coenospecies (? sections) although occasional hybrids may be formed. If, however, there is some degree of sterility in the hybrids within the sections, it might be necessary to revise this provisional biosystematic classification. The absence of fertile seeds in crosses between members of Sect. Vesicariae and other sections does not, of course, imply that no such hybrids are possible since very few combinations of individuals were tested, and much more fertile pairs may exist. Even if natural cross pollination is a rare event, the number of possible combinations which could occur over a period of hundreds or thousands of years is enormous, and the rate of production of hybrid seeds on a world wide basis is probably quite high. It is unlikely that many of these will survive, but there could still be a significant rate of production of hybrid individuals over a reasonably long period on a world-wide scale. In addition, crossing carried out in experimental studies may bear little relationship to the behaviour of the species involved under natural conditions where a wide range of environmental factors may operate to enhance or decrease the probability that hybridisation will occur. The reported natural hybrids used to construct fig. 2.3 are therefore not excluded by the results obtained but a considerable element of uncertainty must remain because it is impossible to show that all the reports are correct. When the critical nature of many Carex species is considered, errors in identification might be expected.

Within the Vesicariae, the evidence available at present does not allow any firm conclusions to be drawn on the relationships of the species. On the basis of seed set, it would appear that C.grahamii can cross extensively with both C.vesicaria and C.saxatilis, while these two species give a lower fertility when crossed. This would agree quite well with a hybrid origin for C.grahamii, but it is also noticeable that this species also gives high fertilities when crossed with C.rostrata, a species which is, in the light of cytological and morphological evidence, unlikely to be implicated in the immediate ancestry of C.grahamii. In general, I believe that the use of seed set figures as the sole means of estimating the degree of relationship between species is rather unsatisfactory since it is very difficult to obtain consistent results and there are wide variations between individuals and replicates of the same cross. Even when a large number of replicates has been made and the data treated statistically, it is by no means certain that a species pair showing high seed sets in crosses are any more closely related than a pair in which fertility is very low. It is entirely possible, if not likely, that two closely related species which frequently occur together in similar habitats would develop stronger interspecific barriers than a pair of less closely related species which are effectively isolated by ecological or geographical barriers. In the case of Carex species (e.g. C.rostrata and C.vesicaria), it might be expected that barriers would appear at the pollen/stigma/style interaction stage, since the wind pollination system which occurs in the whole genus is indiscriminate and some form of elimination of foreign pollen would be advantageous. Eaton (1973) considers that 'seed incompatibility' is probably not a good indicator of phylogenetic relationships, in Primula at least.

Similar objections of course apply to the use of later stages of gene exchange between species, but chromosome pairing and the fertility of hybrids and backcrosses probably present a more accurate interpretation of genetic affinity than would ever be possible from seed set. Unfortunately, at present seed set is the only indicator available and the growth of hybrid plants gives no information: so far, all appear to grow equally well. The pattern of fertility relationships observed in Sect. Vesicariae in many ways resembles the 'Ceanothus pattern' described by Grant (1971, p.100) in that species are usually interfertile within sections and are probably separated by ecological preferences while strong crossability barriers exist between sections. Pollination is 'promiscuous' and this appears to be the major distinction from the 'Geum pattern' found in perennial herbs in which there are moderate differences in floral mechanisms. It would seem that in many of their breeding characteristics, Carex species may closely resemble the long-lived trees with outbreeding systems. This is not entirely unexpected since in the longevity of their vegetative clones, comparatively specific ecological requirements and limited areas of colonisation by new individuals, Carex species share many biological characteristics with trees and therefore tend to have outbreeding systems which encourage diversity and intense selection.

CHAPTER 8 MORPHOLOGY

8.1 Introduction

The main aim of the studies described in this chapter was to investigate the variation patterns occurring in members of Sect. Vesicariae with particular emphasis on C.rostrata. This species was examined in some detail as numerous specimens were available and a large natural population was present locally and was easily accessible. An attempt was made to study variation at different taxonomic levels in order to give some indication of the relative importance of different components in the overall variation ranges of the species. C.grahamii was investigated in some detail and compared with population samples of C.saxatilis and C.vesicaria.

Principal components analysis (PCA), a multivariate statistical technique,,was used to analyse much of the data obtained, since it provides a valuable method of summarising information from a large number of characters in a (usually) smaller number of composite variables which require fewer dimensions for pictorial representation. This technique also has the characteristic that it is not necessary to impose any structure on the data a priori, as in methods such as canonical analysis and discriminant analysis and therefore depends less heavily on the preconceived ideas of the investigator. If the individuals being investigated are represented as points in an n-dimensional space where each of the original variables measured forms one of the axes, the effect of a PCA is to transform the axes such that one of them will be equivalent to the axis showing the maximum variance in the sample. This is the principal axis or first principal component. The second and subsequent principal components represent the orthogonal axes showing the next largest variances (i.e. all the axes are at right angles to each other). The results of an actual principal components analysis are expressed in a series of eigenvalues and eigenvectors. Each principal component has an associated eigenvalue and eigenvector. The former express the proportion of the total sample variance accounted for by each component and because the sum of the eigenvalues is equal to the total variance of the sample, it is therefore easy to convert the eigenvalues to percentage variance. The eigenvectors are made up of a series of multiplication factors required to change the original variable scores into scores on the new composite variables (the principal components). It is usually found that the first few components express a large proportion of the variance in the sample and the relationships between the individuals can thus be represented by only a few (often only two or three) composite variables without serious loss of information. This greatly reduces the number of dimensions needed for pictorial representation, especially if the original variables were numerous. The relative sizes of the elements of the eigenvectors indicates the importance of the contribution made by each of the original variables to the new composite variable. A high positive or negative value for any element means that the original variate multiplied by this element will make an important contribution to the individual scores for the associated principal component. Conversely, if an element of the eigenvector approaches zero, then the contribution made by the corresponding variate will be very small. The theoretical background for the technique is given by Seal (1964) who also gives recommendations for its use. Examples of studies in which PCA has been used are given by Blackith & Reyment (1971).

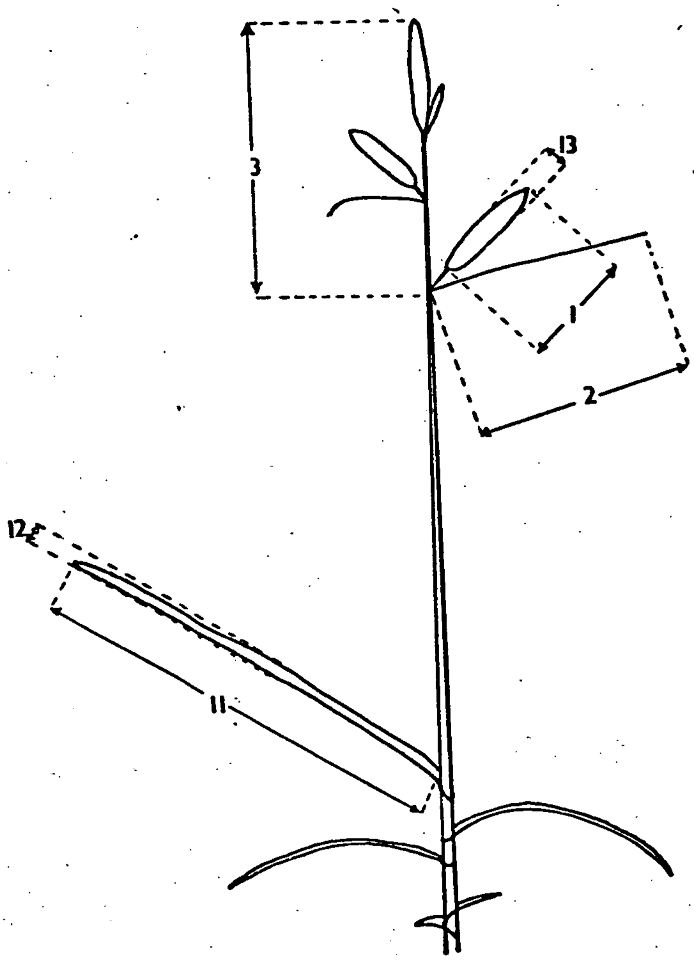
8.2 Materials and Methods

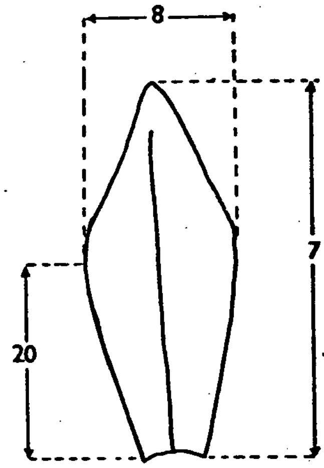
All the morphological measurements were made on dried herbarium material. Population samples were collected at the localities listed in Appendix 1, and additional measurements were made on herbarium material from Edinburgh (E), Kew (K) and the British Museum (BM). Herbarium specimens used are also listed in Appendix 1. A maximum of 22 characters was measured, but in many cases it was not possible to use all of these, especially in herbarium material which was often incomplete and, as a result, only 12 characters were included in the PCA. A list of characters is given in Table 8.1, some of these being further explained in Fig.8.1. The majority were based on normal morphological attributes but a number of epidermal characters were also observed using cellulose acetate impressions as described in Chapter 5. These observations were made for most of the material but were not included in the PCA because they were normally recorded in presence/absence form and not as continuous variables. Some of the results obtained from the use of cellulose acetate impressions were confirmed by Scanning Electron Microscopy (SEM) as described in Chapter 5. Leaf measurements were made on the highest leaf on the culm. In some cases, a 'sterile bract' was present below the inflorescence proper. In C. saxatilis these could be recognised by the dark auricles found at the base of the lamina, a structure which is normally found only on the bracts and not on the vegetative leaves. Such bracts were not used, and only the uppermost of the 'basal' vegetative leaves were measured. Cellulose acetate impressions were made at the mid-point as were measurements of leaf width. The length of the lowermost female spike was measured from the insertion of the lowest floret to the tip of the spike, but where some male flowers were present these were not included. One well-developed

Table 8.1 Characters measured.

1. Length of female spike.
2. Length of lowest bract.
3. Length of inflorescence.
4. Length of utricle.
5. Width of utricle.
6. Length of beak on utricle.
7. Length of female glume.
8. Width of female glume.
9. Length of male glume.
10. Width of male glume.
11. Length of leaf.
12. Width of leaf.
13. Width of female spike (2 x length of utricle)
14. Length of ligule.
15 Width of utricle.
16. Shape of ligule tip.
17. Number of papillae/0.5 mm. length of adaxial leaf surface.
18. Number of papillae/0.5 mm. length of abaxial leaf surface.
19. Presence of silica bodies on abaxial leaf surface.
20. Position of widest part of female glume (Threipmuir material only).

Fig.8.1 Diagram showing location of measurements for morphological studies. Numbers refer to characters in Table 8.1.


3
13
1
2
12
11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7
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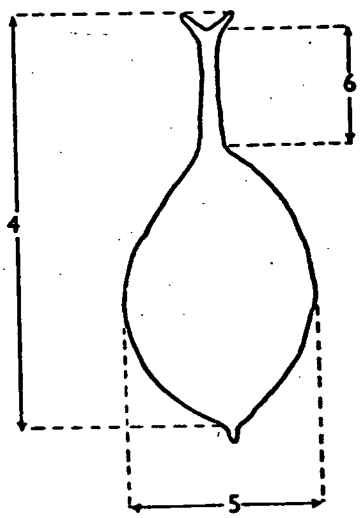
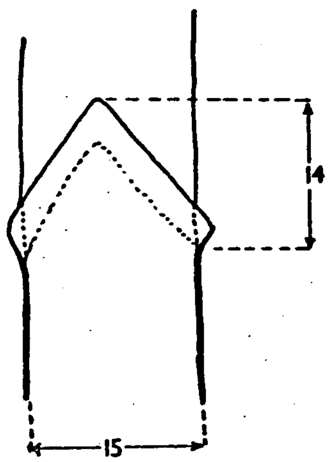

6
4
5


14
15

utricle and its subtending glume were removed from the middle of the spike, since utricles at either end tended to differ in size and shape from the rest. A glume was also removed from the mid-point of the terminal male spike. Glume and utricle characters were measured by projection at known magnification which enabled measurements to be made with a ruler. This was done by sticking the glumes and utricles to a piece of cleared 35 mm. film base with double-sided sellotape, mounting this in a plastic slide mount and projecting through an ordinary slide projector. This method also made it easy to obtain outline drawings. In the case of character 6, the utricle beak length, in some of the utricles examined it was difficult to find an abrupt transition from the beak to the main body of the utricle, and measurements of this character are to some extent subjective although an effort was made to ensure that these were as consistent as possible. A major difficulty in the use of herbarium material was that many of the specimens were incomplete, and had lost parts of the uppermost leaf or the inflorescence, and therefore could not be included in a PCA since missing data are not permitted in the computer programme used. In much of the herbarium material, the ligule could not be examined without damage to the specimens and ligule characters were therefore only measured in population samples.

The morphology of hybrid populations was investigated using the standard pictorialised scatter diagram techniques of Anderson (1949) and PCA in the case of <u>C.grahamii</u>. The Clustan 1B computer programme was used for the PCA and this was run at Edinburgh Regional Computer Centre on an IBM 370/158 computer. The analysis was carried out using a correlation matrix derived from the raw data to standardise the means and variance for all the variables, and was of the standard R-type (i.e. correlations between characters were considered - cf. the discussion on this point by Blackith & Reyment, 1971). No cluster analyses were made on the data.

A nutritional levels experiment was carried out on C.rostrata (sect. 8.3.1) was of a standard Latin squares 3 x 3 experimental design with three nitrogen levels and three phosphorus levels. A diluted (1/5 normal strength) Hoagland solution was used, with the plants grown in sand in polythene lined pots and constantly immersed in the solution as described in Chapter 2. The nutrient levels were:-

1) Low - half the normal Hoagland concentration

2) Normal - standard concentration

3) High - twice the normal concentration

for both phosphorus and nitrogen, giving 9 possible nutrient level combinations. Material of four clones was grown giving a total 4 x 3 x 3 design.

Much of the investigation carried out on clonal differences in C.rostrata was based on material collected at Threipmuir reservoir, Balerno, Midlothian. A large and variable population of this species was present at the site. Several transects were made in different parts of the reservoir during a dry period when the water level was extremely low, allowing easy access to areas normally in deep water. In the transects, collections of 20-30 female spikes were made within 1 m^2 quadrats placed at various intervals along the transect, and these were used for morphological studies. Details of general plant size and cover were noted for each quadrat.

8.3 Variation in C.rostrata

C.rostrata is known to be extremely variable, both on a small-scale local population level and on a wider geographical basis. Studies have been carried out at three main levels:-

1) variation within clones

2) variation between clones

3) variation on a geographical scale

A statistical analysis of the importance of different sources of variation has not been made because adequate data are not available, but a general impression of the contributions made by each can be gained from the results obtained here.

8.3.1 Variation within clones

A certain degree of intraclonal variation is immediately apparent when a single clone stand is examined. There is a considerable change in the overall size of the plants at different water depths, although individual shoots may be quite uniform in the appearance and shape of the component organs. This is very obvious when a transect is taken across different water depths at the edge of a pool. In one transect of this type at Threipmuir, maximum leaf length varied from 40 to 100 cm., when measured from mud level to the tip of the longest leaf, but the plants included in this transect were morphologically very uniform, varying mainly in size rather than shape. Shoot size reached a maximum in intermediate water depths and decreased sharply in very deep or shallow water.

The nutritional levels experiment was intended to test the effects of nutrient levels on the characters used in later morphological analyses. The reasons for the choice of nitrogen and phosphorus as the nutrients to be tested were:-

1) nitrogen normally has a general effect on plant growth rate and size and was expected to alter the stature of the plants and indicate the general size alterations possible.

2) phosphorus is suspected to produce alterations in leaf shape when present in high levels at least in <u>C.nigra</u> (cf. Jermy & Tutin, 1968; p.142). Leaf shape is one of the characters used in other parts of the present study and it was therefore considered important to determine the effects of nutrient level on this character.

The plants grown in this experiment could not be induced to flower and it has not been possible to determine the effects of nutrient levels on floral and inflorescence characters. Changes in vegetative morphology appeared to be rather limited. There was a considerable difference in leaf size and in general growth of the plants between the combined low N & P and the higher levels, with average leaf lengths of ca. 25 and 40 cm. respectively and the corresponding numbers of shoots/plant were ca. 5 and 12-18. The effect on leaf shape, however was very slight and did not appear to be significant. No statistical analysis was carried out partly because only vegetative characters were available, and partly because the differences involved were so trivial.

The above observations suggest that one of the major effects of environmentally generated intraclonal variation is to increase the proportion of the variation attributable to size differences, but variation in shape is less affected and it is therefore probably preferable to use shape differences in comparing species as far as possible. However,the problem of intraclonal variation requires rather more detailed study and it is probable that a more extensive survey of nutrient levels using a wider range of nutrients would reveal a greater susceptibility to environmental factors than the present studies suggest. The effects of water level also need to be examined experimentally, along with pH and light intensity.

8.3.2 Interclonal variation

In the course of seed collection and general observations of C.rostrata populations at Threipmuir, it was noted that extensive differences in morphology occurred in plants growing in different parts of what appeared to be a homogeneous habitat. A number of transects were therefore laid out, collecting material from a site where a single uniform population appeared to be present (transect I) and from a rather more heterogeneous area where several morphological types occurred. Transect II was positioned to cross a boundary between two of these morphological types, so that each end of the transect lay in a different type while the boundary between the two occurred around the mid-point of the transect. Although the data from both of these transects have not yet been fully extracted quadrats analysed from transect I and transect II (nos. 1, 4 & 10) show interesting results. Measurements were made on utricle and glume characters (characters 4-8 and 13) for 20 individual spikes from each quadrat. From these data, it would appear that three fairly distinct forms can be separated on a morphological basis. It is assumed that these are different clones. The three forms involved, labelled 1 (transect I), 2 (transect II, quad.1) and 3 (transect II, quad.10) were very distinctive, showing well-marked differences in female spike structure as can be seen from Fig.8.2. There were also differences in utricle and female glume shapes and a PCA was carried out on measurements of these organs. Examples of glumes and utricles are shown in Fig.8.2. From the histogram of utricle length (Fig. 8.3), it is obvious that although there is considerable overlap, the three clones are distinctive and clones 1 & 3 in particular can be distinguished almost completely on the basis of utricle length. In this case, a PCA using a correlation

Fig. 8.2 Clonal variation in C.rostrata.

a) examples of female spikes from clones 1, 2 & 3.

b) utricles and glumes from clone 2

c) utricles and glumes from clone 3

Fig. 8.3 Clonal variation in C.rostrata

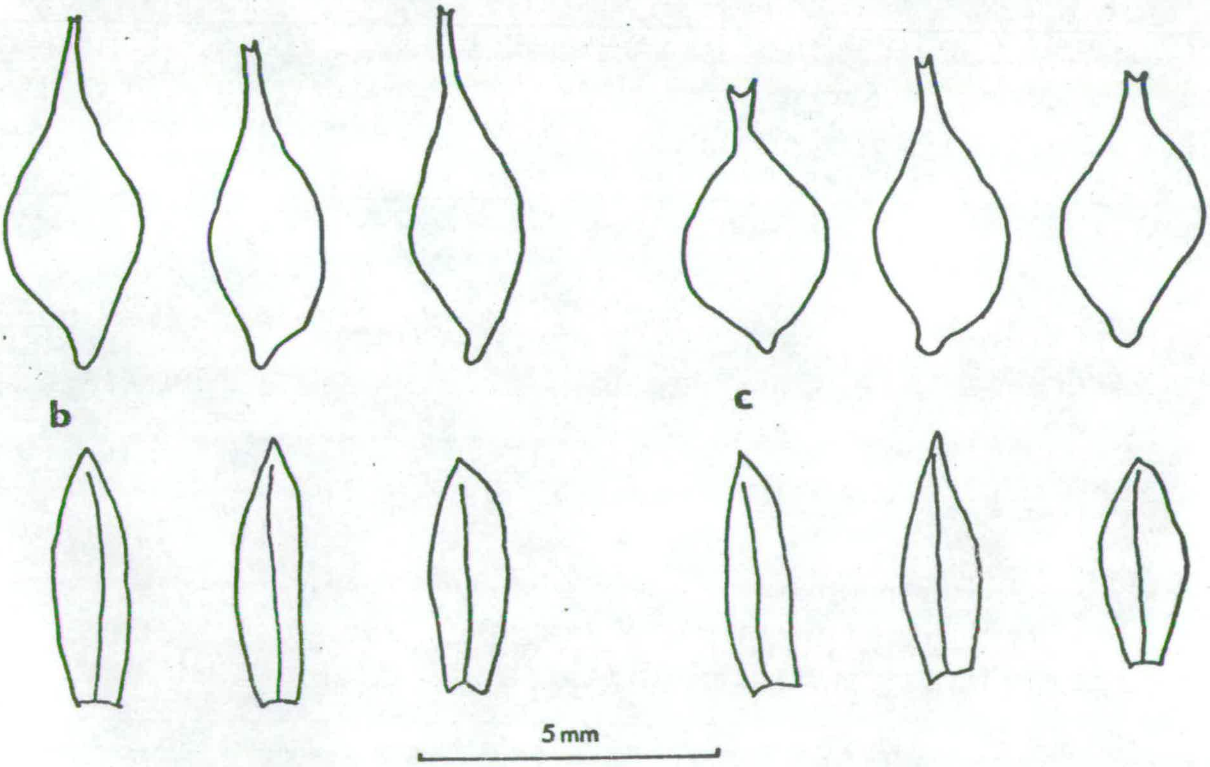
a) histogram of utricle length

b) histogram of scores for component I of PCA

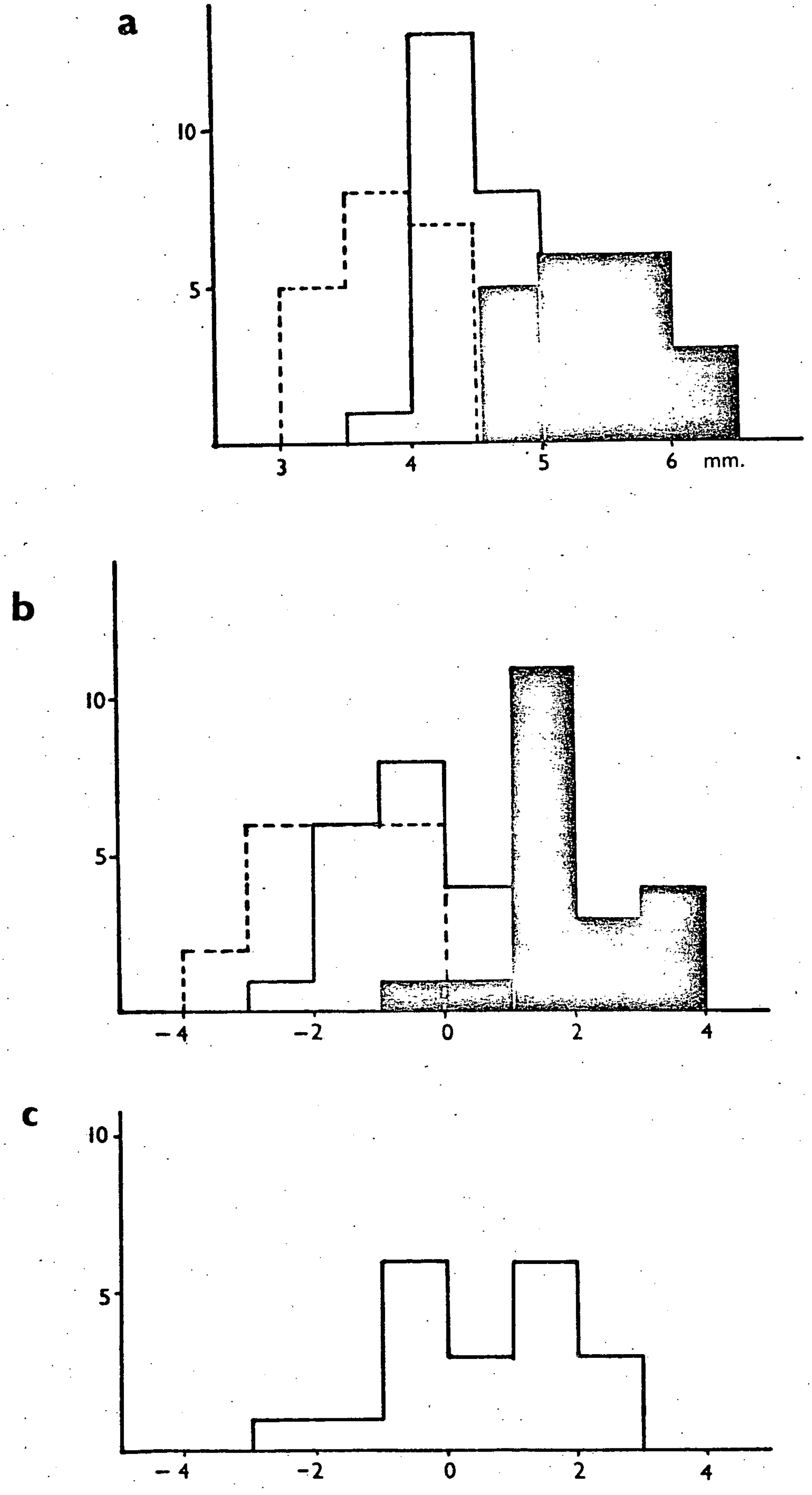
c) histogram of scores for component I of PCA for quadrat II.4

clone 1 clone 2 clone 3

a



8.2



8.3

matrix did not show much better separation between clones than the simple unmodified utricle length character, but the first component separates clones 2 and 3 more clearly (Fig.8.3). Quadrat II.4 was situated in the middle of transect II on what appeared to the eye to be the boundary between clones 2 and 3. When the scores for component I are considered for the material collected from this quadrat, it can be seen that a distinctly bimodal distribution is produced (Fig.8.3) with peaks corresponding to the maxima for clones 2 and 3. It appears therefore that two distinct forms were present in this quadrat rather than a mixture of intermediate plants. It is possible that the use of a variance/covariance matrix might produce clearer results than a correlation matrix in this case, as the variances of most of the characters are fairly similar (cf. Seal, 1964). This option, however, is not available in the 'Clustan' programme.

The possibility that the morphological variation encountered in the Threipmuir transects was caused by environmental factors cannot be eliminated, as no cultivation tests have been carried out on the clones to determine the stability of the characters used. In spite of this objection, however, a genetical cause seems most likely for the following reasons:-

1) The habitats examined were relatively uniform, consisting of gently sloping or level mud around the edges of the reservoir. No obvious differences in substrate were visible.

2) The observed morphological discontinuities were quite sharp, and at the boundaries, a mixture of different plant types were present, rather than a gradual transition from one to the other.

3) The discontinuities did not run perpendicular to any obvious environmental gradient such as water depth, but instead tended

to cut across different water depths. In transect I, where a complete range of environmental factors from dry land to deep water was present, there was no obvious discontinuity and variation was mainly in plant size.

If environmental factors are responsible for the morphological variation observed, they are probably subtle interactions of substrate and other factors which together produce profound alterations in morphology. This seems rather unlikely in view of the apparent uniformity of the plants in transect I over a wide range of environmental changes, and it therefore seems preferable to postulate a genetical basis for the variation pattern described for the C.rostrata population at Threipmuir Reservoir.

It would appear, therefore, that it is possible to distinguish morphologically between some clones of C.rostrata, and in certain cases this can be done visually in the field if the environment is reasonably uniform. Variation between clones is therefore probably a major component of the variation range of this species. Observers may tend to overemphasize the significance of such variation, partly for psychological reasons, since when faced with a large and morphologically uniform clonal stand they are likely to notice and pay particular attention to any discontinuity occurring between different clones. If single representative shoots from a large number of clones were presented, the discontinuities would appear less abrupt and the situation would closely resemble a continuous variation range. The clonal population structure in C.rostrata may therefore tend to make us exaggerate the importance of morphological variation within a small geographical area. In spite of these reservations, it is nevertheless obvious that this species does have an extremely wide variation range - this is immediately apparent when a selection of material from a large herbarium is examined.

The type of variation noted here appears to be common to several species with vigorous means of vegetative propagation. The approximate size of the clones found at Threipmuir was at least 20-30 m. and possibly considerably larger. A growth rate of ca. 20-30 cm. per year at the edge of a colony seems possible from field observations of rhizome lengths, and if such a growth rate is assumed for a circular colony growing constantly, a clone cf size 30 m. diameter would be approximately 50 years old. This is probably a minimum estimate since no allowance is made for slow growth in the early stages or for limiting effects of competition. Growth rate and clonal size need to be investigated in more detail, however, before any accurate estimate of clonal ages can be made. Harberd (1961, 1962) concluded that some clones of *Festuca* spp. which he investigated were possibly as much as 1,000 years old and extended for at least 250 yards, but in these species, pure stands are uncommon and the individual shoots are usually mixed with other species. The situation in *C.rostrata* more closely resembles that in *Phragmites communis* which also tends to grow in dense stands along the edges of ponds and rivers. Björk (1967) found good evidence of well marked clones occurring side by side, but these were at different ploidy levels and were very distinct in their morphology. No direct chromosome counts were made on the Threipmuir transect specimens, but all the counts made earlier for a number of plants in the same part of the reservoir gave the same ploidy level (Ch. 7). There are many strong resemblances between these two species as both often grow in similar habitats in dense stands, and appear to form extensive single clones by vigorous vegetative reproduction. Like

C.rostrata, Phragmites frequently has a rather low seed set - Gustafsson and Simak (1963) reported seed sets ranging from 0 to 10% - and seedling establishment appears to be comparatively rare (Haslam, 1972). Its ecological range is, however, wider and its distribution range is much larger (Haslam, op.cit.).

8.3.3 Regional variation

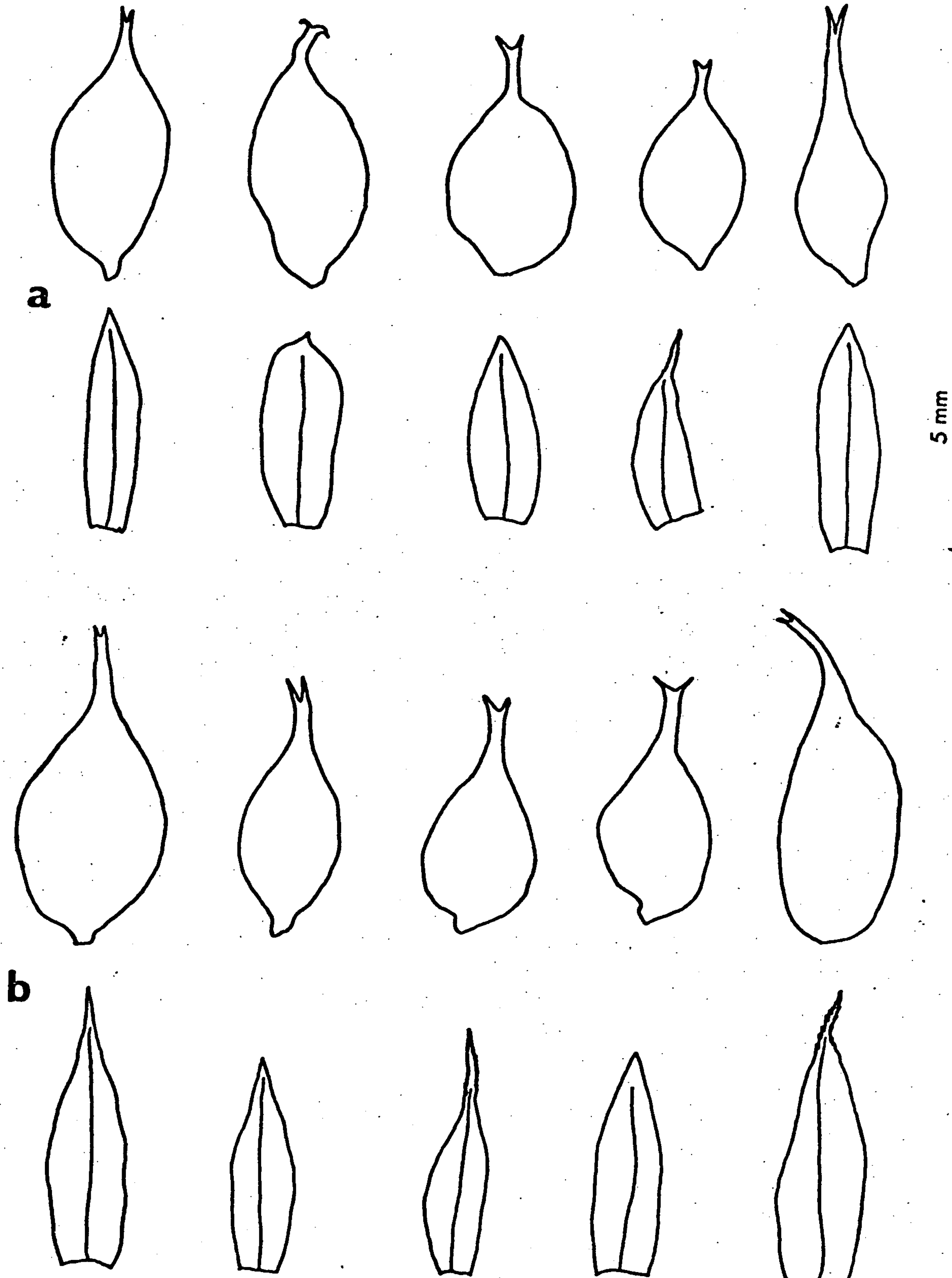
The morphological variation described in the previous section appears to be more or less random and operates over quite a small scale (10-100 m.) but larger scale variation patterns which are correlated with geographical distribution can also be found. One of the best examples occurs in the variation noted for the N.American part of the distribution range. A number of authors have pointed out that American material of C.rostrata differs from European forms in a number of details cf. fig. 8.4. These differences led Boott (1840) to describe the American form as a separate species - C.utriculata Boott. The majority of later authors have taken the view that this form should be treated as a variety - var.utriculata (Boott) Bailey - and not as a separate species. The most extreme position was taken by MacKenzie (1931) who regarded the variation observed in C.rostrata as being of no taxonomic significance and recognised only a single species without any subspecific divisions. Possible reasons for MacKenzie's decision are given by Fernald (1942), who considered that MacKenzie's failing eyesight made some of his later taxonomic decisions unreliable.

In the course of examining a range of herbarium material, it became obvious that further investigation of N.American forms was required. A number of specimens (Appendix 1) were studied in greater detail and epidermal and general morphological characters were examined. A PCA was carried out on data from morphological characters 1-12 and

Fig.8.4 Examples of utricles and glumes from N.American variants of *C.rostrata*.

a) papillate, slender forms

b) var.*utriculata* (non-papillate)


a
5 mm
b

data obtained from a population of C.rostrata collected at Tamdhu, Moray, Scotland were included as a sample of the European type variety.

The first two factors in this case accounted for 56% of the total variance in the sample. Component I appears to be primarily concerned with the general size of the plants as all the elements of the first eigenvector are positive, so that larger plants have the highest scores, while the second and subsequent components are indicators of shape since their eigenvectors contain a mixture of positive and negative terms. This agrees with the 'normal' pattern described by Blackith & Reyment (1971), where the first component very often reflects size variation in the sample. The eigenvectors and the associated eigenvalues and percentage variance are listed in Table 8.2.

The results of the analysis for components I and II are shown in Fig.8.5. It can be seen that with the exception of only one individual, all of the Tamdhu population is contained within the lower/left hand half of the diagram while the N.American material is situated almost entirely in the upper/right hand half. Neither component separates the two groups completely, but the combination of the two produces a distinctive distribution of the material with very little overlap. There is also a slight separation of the two groups on component III which accounts for 12% of the total variance, but the separation is not very complete as can be seen from Fig.8.6. The characters receiving the heaviest weightings were as follows:

Component I

High scores for plants with - wide leaves, long bracts, long inflorescences, wide utricles and long leaves.

Table 8.2 Eigenvalues, percentage variance and eigenvectors from PCA (*C. rostrata*/var. *utriculata*) (only the first 6 eigenvectors are shown).

EIGENVALUES

4.53	2.23	1.45	1.21	0.66	0.60	0.43	0.30	0.22	0.17	0.11	0.08

PERCENTAGE VARIANCE

37.74	18.61	12.11	10.12	5.49	5.04	3.55	2.54	1.87	1.40	0.90	0.69

CUMULATIVE VARIANCE

37.74	56.35	68.45	78.56	84.05	89.08	92.63	95.17	97.03	98.43	99.32	100.00

EIGENVECTORS - BY ROWS

VECTOR 1	0.087	0.374	0.362	0.294	0.342	0.134	0.259	0.278	0.160	0.214	0.325	0.422
VECTOR 2	-0.566	-0.210	-0.304	0.186	0.339	0.200	-0.207	0.420	-0.060	0.285	-0.222	-0.005
VECTOR 3	-0.028	-0.178	-0.104	0.350	-0.143	0.537	0.337	-0.072	0.515	-0.304	-0.034	-0.221
VECTOR 4	-0.201	0.277	0.066	-0.090	-0.008	0.473	-0.399	0.015	-0.342	-0.478	0.376	-0.027
VECTOR 5	-0.101	0.076	0.279	-0.462	0.196	-0.028	-0.404	0.041	0.688	-0.045	-0.096	-0.055
VECTOR 6	-0.412	0.142	-0.183	0.342	-0.091	-0.260	-0.206	-0.571	0.225	0.177	0.355	0.090

Fig.8.5 Scatter diagram for components I & II of the PCA carried out on _C.rostrata_ and _C.rostrata_ var. _utriculata_.

(□) _C.rostrata_ (Tamdhu population)

(◇) _C.rostrata_ var. _utriculata_

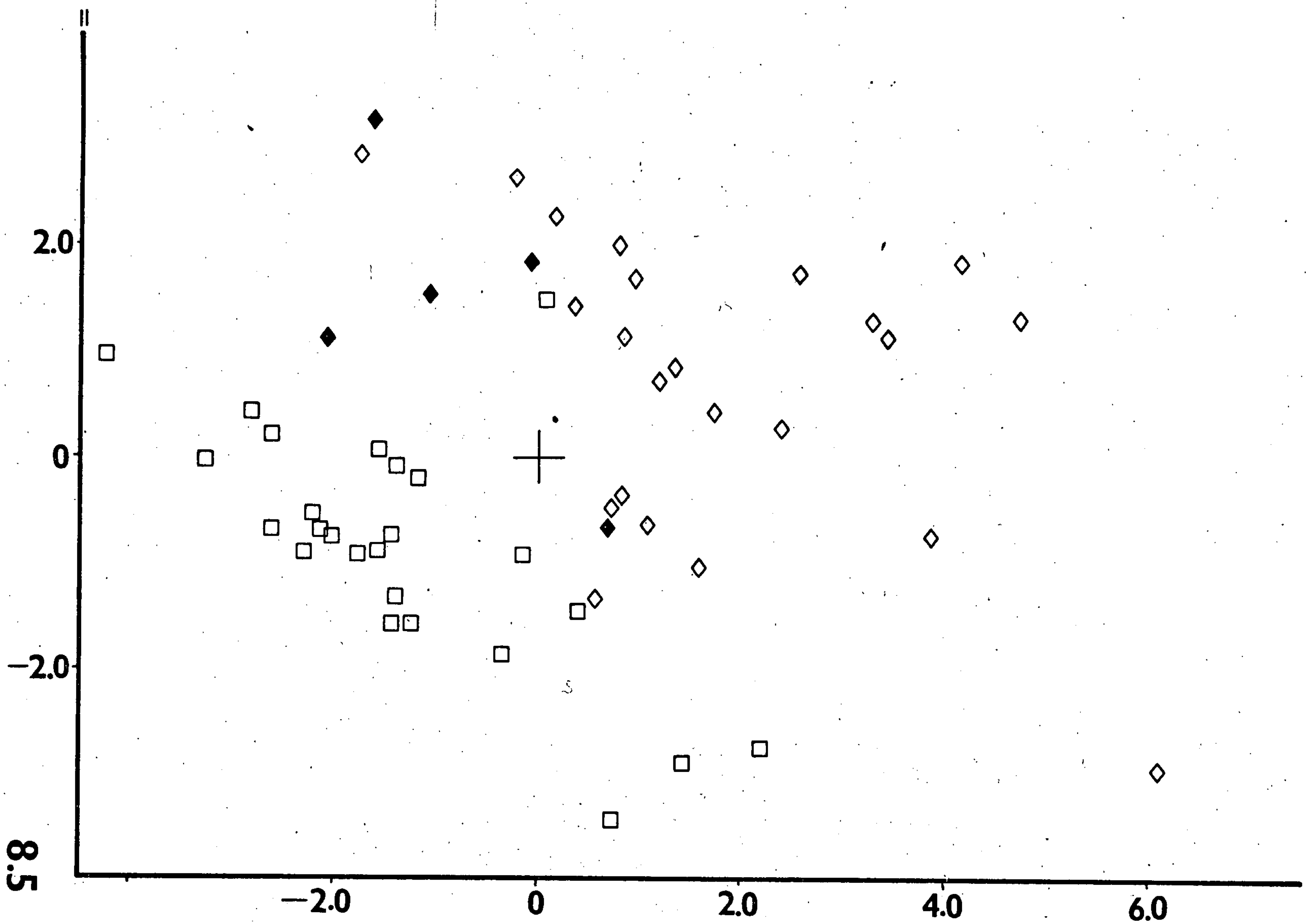
(◈) papillate N.American forms

Fig.8.6 Scatter diagram for components I & III.

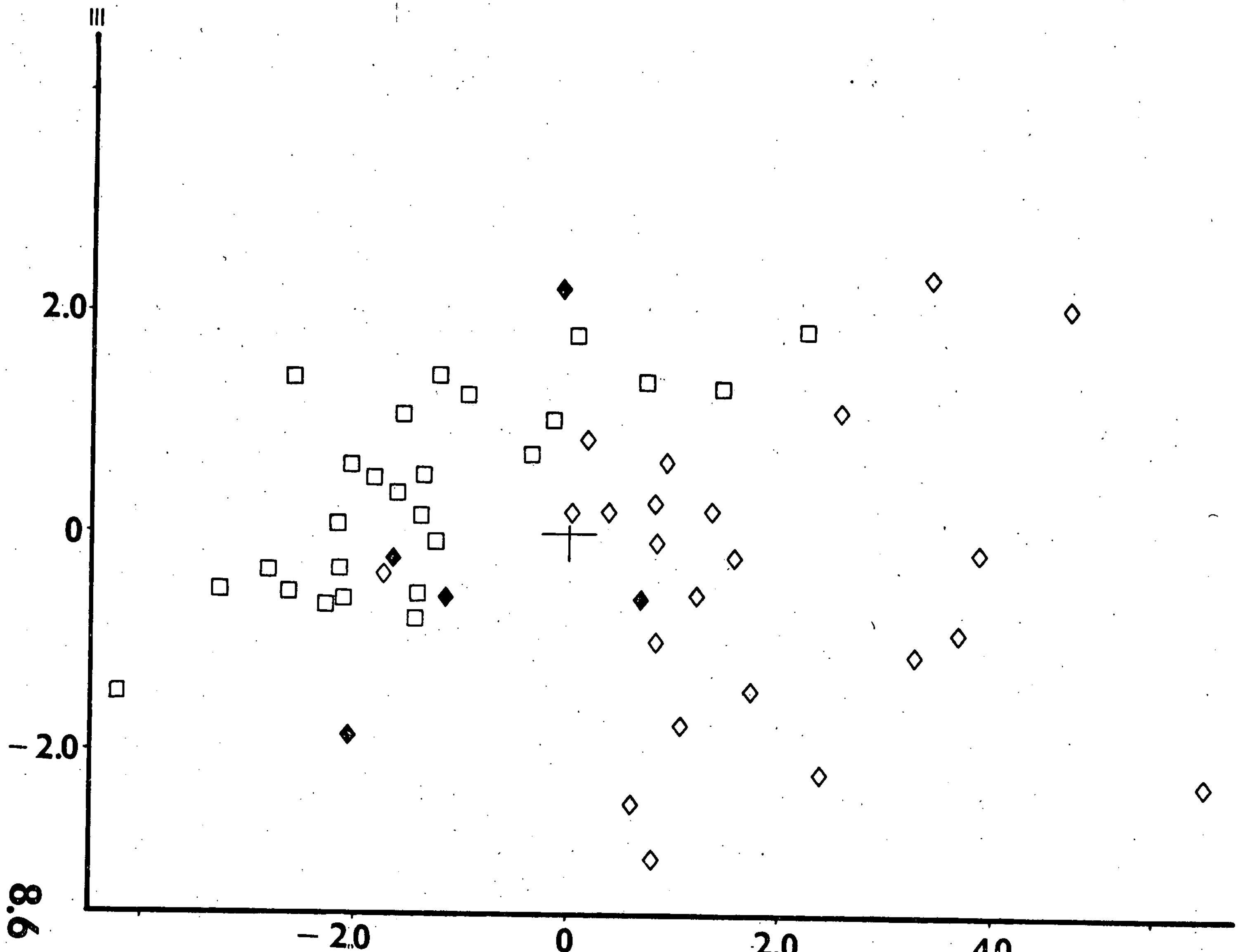
symbols as above.

Fig.8.7 Distribution of N.American specimens included in PCA.

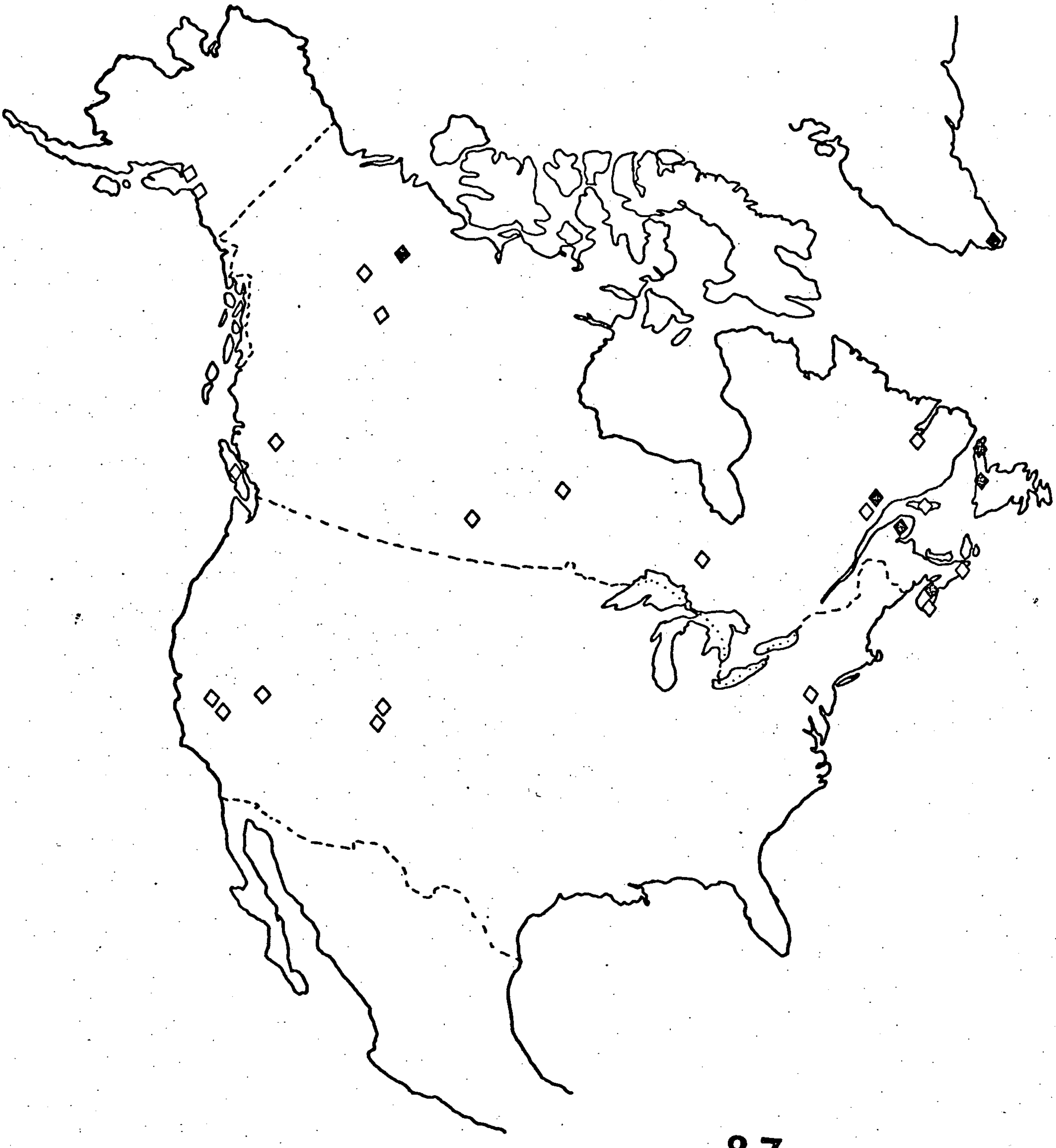
symbols as above.



8.5



8.6



8.7

Component II

High scores for plants with - short female spikes, wide female glumes, wide utricles and short inflorescences.

Component III

High scores for plants with - long beaks on the utricle, long male glumes, long utricles, long female glumes, narrow male glumes.

The characters are arranged in descending order of weighting, and plants receiving low scores will of course have the opposite combination of characters for each component. These three components together account for almost 70% of the total variance of the sample. It is obvious that the first component does not lie exactly along the axis giving maximum separation between European and N.American forms, and instead tends to reflect both the differences between the forms in their general size (var. utriculata is usually considerably larger) and the strong size variation occurring within each group. The most efficient method of finding the optimum characters to distinguish between var. utriculata and the type variety would be to use discriminant functions (cf. Blackith & Reyment, 1971) but this technique has the disadvantage that it is necessary to impose a structure on the data a priori by dividing the material into two or more groups and searching for the characters producing the maximum distinction between the groups. A PCA does not require a preliminary separation into groups and is therefore to a certain extent more objective, but the resulting principal components may not correspond very closely to the 'best' separation possible between the groups. In this case, PCA has proved to be useful as a technique for preliminary investigation and appears to demonstrate a definite structure in the data. For a more detailed study where the optimum characters for separation of taxa are required, discriminant functions and similar techniques should provide the most productive approach, after an initial PCA.

It is difficult to select a set of characters which consistently separate the two forms because, although they are differentiated both by general size and a number of shape characters, considerable overlaps occur. The most useful characters appear to be the overall size of the plant, leaf width, width of utricles and female glumes, relative lengths of the female spikes and inflorescence, length of the beak on the utricle, length of the male glumes and the general utricle size. Var.utriculata is normally larger than the type variety in almost all its characters.

It is also of interest to examine the distribution of epidermal characters in N.American material. All the European material of C.rostrata examined in the present survey had prominently papillate adaxial epidermal surfaces, with adaxial stomata and virtually no silica bodies visible in cellulose acetate impressions. In sharp contrast, the American material consists largely of plants with smooth epidermal surfaces, with abaxial stomata and moderately or highly prominent silica bodies. These differences are illustrated in Fig.8.8. The few papillate individuals found amongst the var.utriculata material showed two main characteristics -

1) They occurred almost exclusively in the north and north-east of the continent, in Canada.

2) They were much more slender and more similar to the type variety than the majority of the var.utriculata material.

The geographical distribution of the papillate and non-papillate plants is shown in Fig.8.7, where the pronounced north-eastern trend shown by the papillate forms is obvious. This is in agreement with statements by Fernald (1942) and Boott (1840) who considered that plants resembling the European forms occurred only in the north and high mountain areas.

The morphological affinities of the papillate N. American forms can be seen from Figs. 8.5 and 8.6 where they fall in a position which is more or less intermediate between the utriculata and European forms on axes I & II, and overlap considerably with the European material on axes I & III. They are, therefore, closest to the type variety in their general morphology, although they do show some departures from the range of the European forms, mainly in the shape characteristics expressed by factor II. In size, they correspond very closely with the European form. It must, however, be borne in mind that the overall sample size is very small. A more extensive survey would almost certainly demonstrate a greater degree of overlap between the groups mentioned here. With this reservation in mind, it would appear that the N.American material of C.rostrata can be divided into two fairly distinctive forms. The first is a small slender plant with adaxial stomata and a papillate adaxial epidermis which closely resembles the typical European variety and appears to be confined to the northern parts of the N.American continent. The second form is a plant corresponding to Boott's C.utriculata which is usually much larger, with wide leaves, abaxial stomata and smooth epidermal cells. This agrees with Fernald's treatment (Fernald, 1942), and two of the gatherings cited by this author as belonging to var. rostrata were found to have a papillate epidermis. The other specimens cited have not been examined. Examples of utricles and glumes of papillate and non-papillate forms are given in Fig.8.4.

On morphological, anatomical and cytological grounds (cf. Chs. 5 & 6), there would appear to be a considerable body of evidence suggesting that the two forms of C.rostrata are of some taxonomic significance and are worthy of formal recognition. The major problem lies in deciding the most appropriate rank to be applied. The varietal rank proposed by Fernald (1942, 1950) seems inadequate since this is now normally applied, in Europe at least, to local variants which do not have a broad, regional distribution pattern (cf. Davis & Heywood, 1963) and it would therefore seem preferable to use subspecific or specific rank for the plant at present known as C.rostrata var.utriculata (Boott) Bailey. This problem will be further discussed in Chapter 9.

The var.utriculata form also occurs in E.Siberia (specimens seen at Kew - Appendix 1) and reaches at least as far as Kamchatka and probably further west. Kreczetowicz (1936) records C.utriculata Boott from European Russia eastwards, but all the broad-leaved specimens of C.rostrata which I have seen from W. and N.W. Europe have a papillate adaxial epidermis and adaxial stomata and it therefore seems preferable to regard these as belonging to C.rostrata s.s., with genuine var.utriculata confined to Siberia. The type variety, with narrow leaves, occurs in Siberia in Tjumen, Kamchatka and Krasnoyarsk, and it seems probable that this form is genuinely circumpolar, extending along the northern parts of Siberia and N.America. I have not seen sufficient herbarium material to enable useful mapping of the ranges of the two forms in Asia.

Kükenthal placed all the European broad-leaved forms in var.utriculata, but as in the Scandinavian and other European specimens have papillate epidermal cells and abaxial stomata. In addition, the leaves are rarely strongly septate-nodulose as in the N.American material, while the female glumes are usually obtuse to slightly acute and never aristate as in many specimens of var.utriculata. I therefore consider that all the European variants included by Kükenthal in var.utriculata should in fact be regarded as being quite separate from it and that the taxon should include only the N.American and Siberian forms with smooth epidermal cells and abaxial stomata. If the European forms are to be given any formal recognition, the correct name would appear to be C.rostrata var.maxima Andersson or one of the many varietal names applied to large robust European forms of C.rostrata. The nomenclature is, however, rather complex and I do not intend to make any formal proposal on the nomenclature of these forms at present. The exact status of the European broad-leaved variants is uncertain. The specimens from W.Ireland have a number of characters which are similar to those of C.laevirostris, e.g. their large size, broad leaves, congested female spikes and the presence of a hypodermal layer in the leaf, but they are identical with typical C.rostrata in many other characters such as the papillate adaxial epidermis and adaxial stomata. It is possible that some of these forms may be relict hybrids (? C.rostrata x laevirostris), similar in origin to C.recta (Faulkner, 1972), but this problem needs to be investigated in more detail.

A further problem occurs in parts of the Southern range of the var.utriculata form in Siberia and Asia, since specimens from Altai and Mongolia appear to be morphologically rather distinctive, often with very large, inflated utricles and dense spikes. It is possible that a complex of closely related species or subspecies occurs in Asia but at present there is insufficient material or information to be able to make any reasonable assessment of the status of these forms.

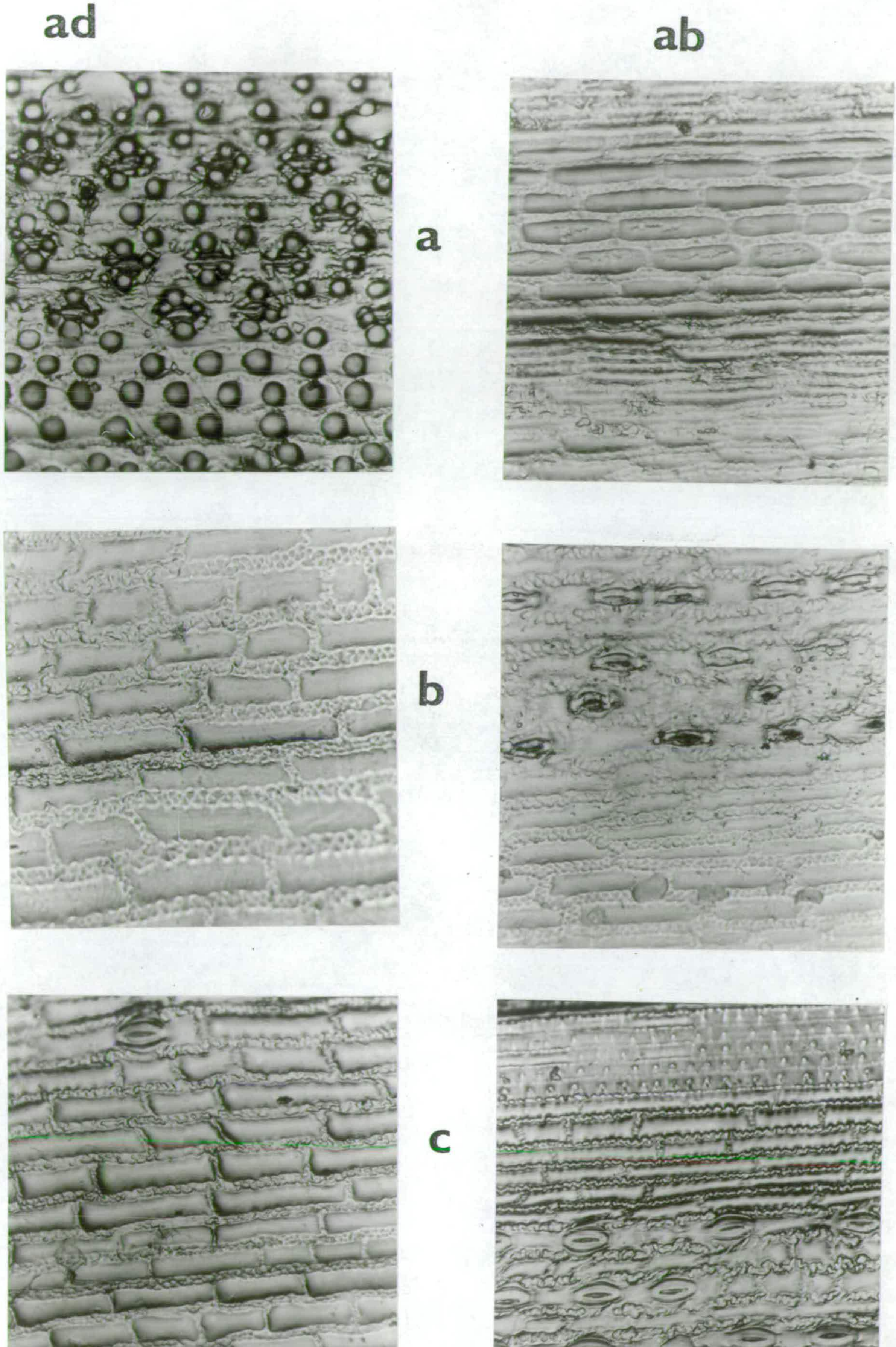
Fig.8.8 Epidermal structures from cellulose acetate impressions.

a) C. rostrata

b) C. rostrata var. utriculata

c) C. vesicaria

ad. adaxial   ab. abaxial.
(x 225)


ad
ab
a
b
c

8.4 Natural hybrids in Sect.Vesicariae

Natural hybrids have been reported very frequently in Carex and within Sect.Vesicariae almost all the possible hybrid combinations have been reported, in addition to numerous intersectional hybrids. Drury (1958) reports that three species - C.rostrata, C.rotunda and C.membranacea - all form hybrids in Alaska, and that one of the hybrids has given rise to a new species (C.paludivagans). Numerous hybrids are reported by Kükenthal (1909) and other authors. Since hybrids appear to be very common, introgression must be considered as a possible source of variation in Carex species, and it was therefore decided to investigate some examples of hybrids more closely in an attempt to determine the degree of introgression possible under natural conditions.

8.4.1 Hybridisation between C.rostrata and C.vesicaria

A site thought to contain natural hybrids of C.rostrata and C.vesicaria (M. McCallum Webster, personal communication) was investigated and a sample of 30 plants collected. This site was a series of small ponds near Grantown on Spey, Moray (V.C.95), which contained both species in the shallower water near the edges of the ponds. These were artificial and obviously subject to a certain amount of human interference and did not form a natural habitat. C.vesicaria in this case was growing in the open and not in the slightly shaded habitats in which it normally occurs. The standard set of morphological measurements was made and the epidermal structures of the specimens examined by means of cellulose acetate impressions and S.E.M. The data obtained have been plotted on scatter diagrams. Fig.8.9 shows pure populations of C.rostrata and C.vesicaria, the former from Tamdhu, Moray, and the latter from Loch Tay. The two species can

Fig. 8.9 Scatter diagram - C.rostrata and C.vesicaria.

axes are LL/LW - leaf length/leaf width

SL/2UL- length of female spike/2 x length of utricle

1. adaxial papilla	>20	10-20	<10
2. abaxial papilla	≥10	present (<10)	0
3. ligule length/width	-	≤1.25	>1.25
4. width of ♂ glume	-	≤0.11	>0.11
5. length of ♀ glume	-	≤5. 5	>5. 5

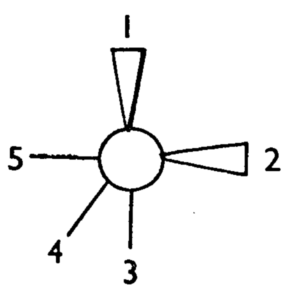
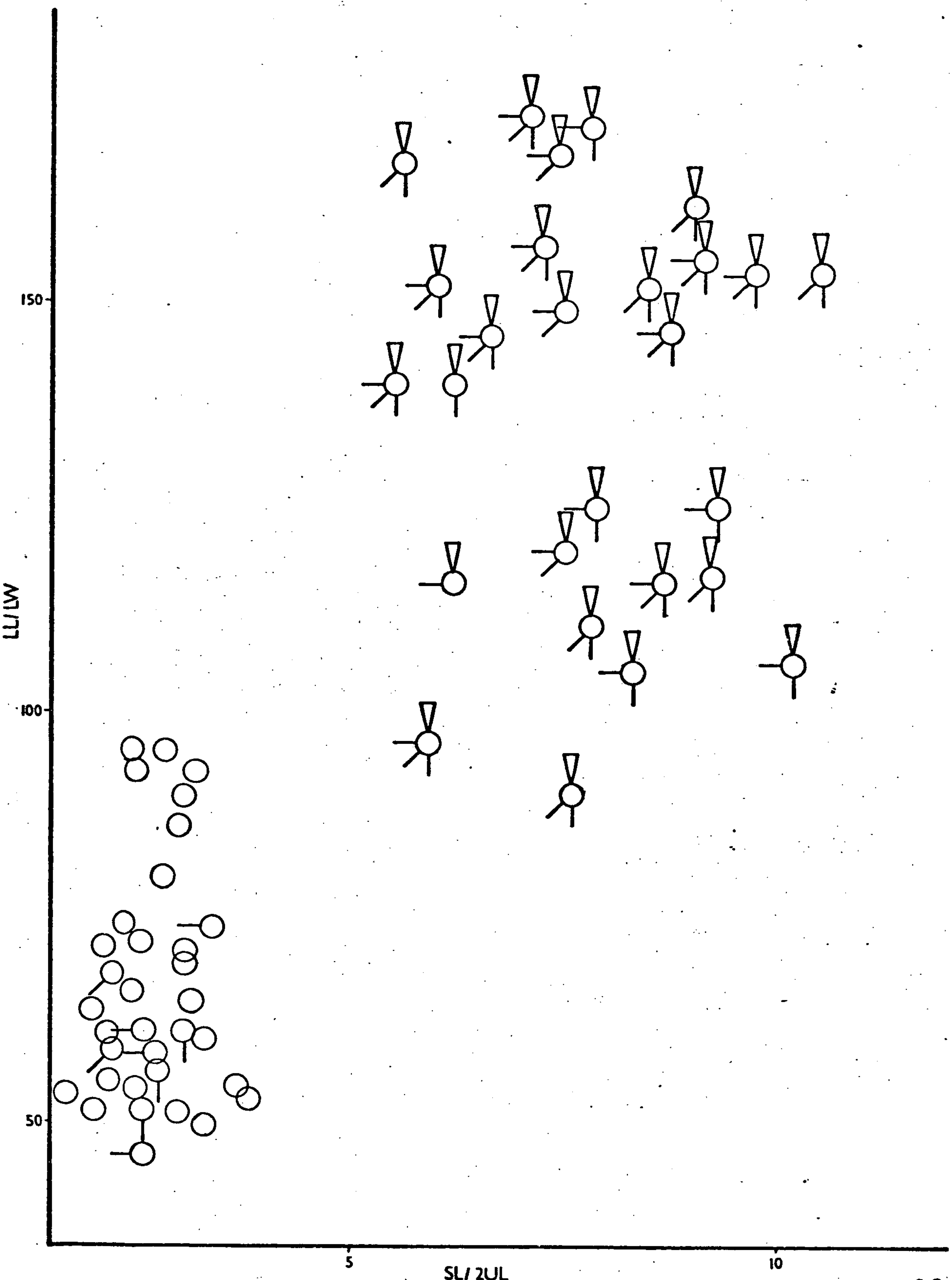


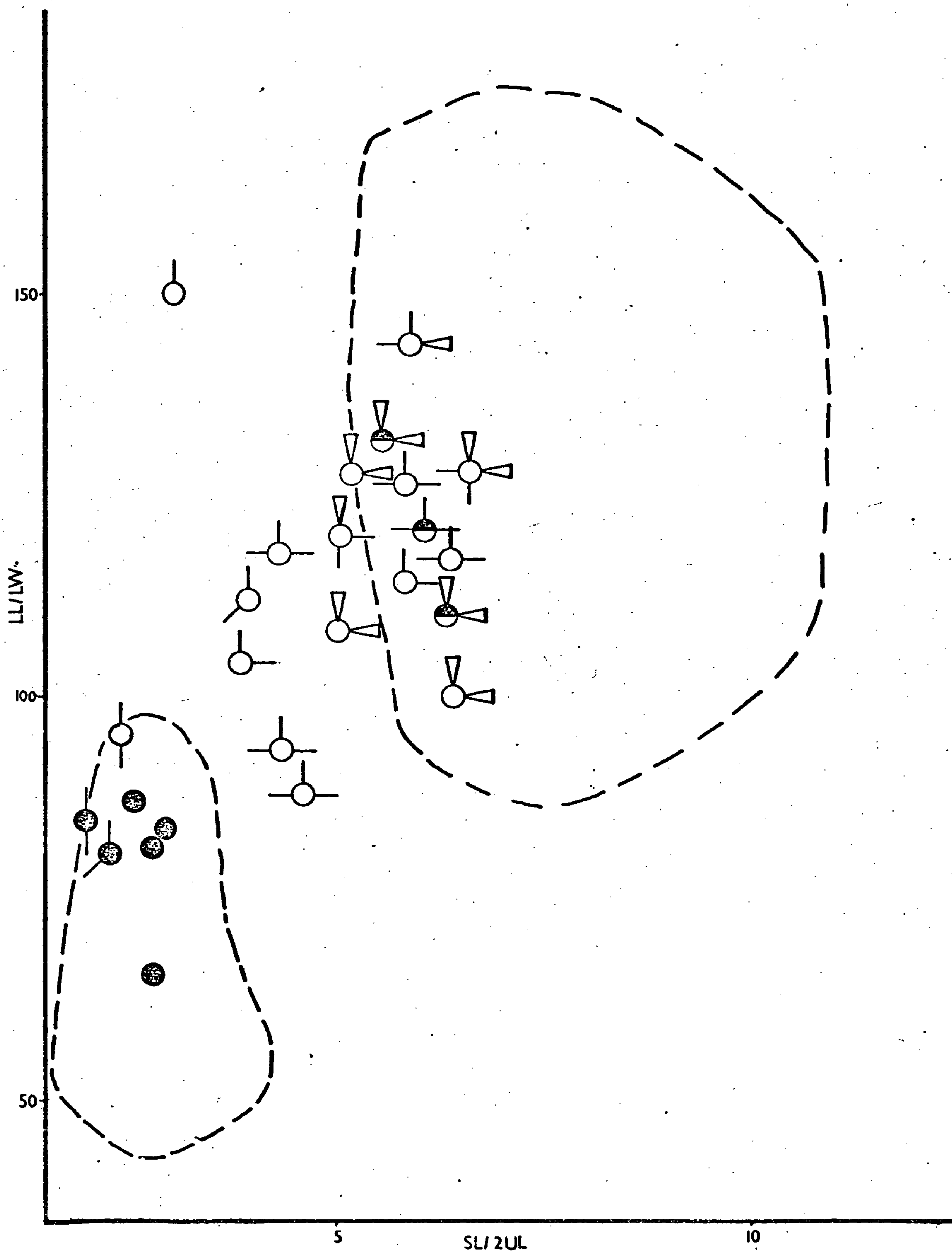
Fig. 8.10 Scatter diagram - Grantown population

symbols and axes as above except

(O) sterile (⊖) a few seeds produced (⊙) fertile



8.9



8.10

easily be separated by using ratios of length of female spike/2x utricle length (equivalent to length/width of the female spike) and length/width of the leaves as the axes, and the other characters shown by the metroglyphs help to give a clear distinction between the species. When the data for the Grantown population are plotted on the same axes (Fig. 8.10), it is immediately obvious that a large proportion of the plants in the sample occupy an intermediate position on the scatter diagram, lying more or less directly between the two species. In addition, many of the other characters indicated by the metroglyphs show that the plants are intermediate. There is another group of plants in the sample which are more or less indistinguishable from typical C.vesicaria material. Morphological evidence would therefore suggest that a considerable proportion of the specimens collected at Grantown were hybrids between C.vesicaria and C.rostrata. This evidence is supported by detailed examination of the epidermal structure. C.rostrata almost invariably has a densely papillate adaxial epidermis (figs. 8.8, 8.11), while nearly all the C.vesicaria specimens examined had a completely smooth adaxial epidermis (figs.8.8 and 8.11). The putative hybrid material had an epidermal structure which was almost exactly intermediate, with papillae present, but less dense and smaller than in C.rostrata (Fig.8.11). The structure and position of the stomata also shows the intermediate nature of the putative hybrids as can be seen from fig.8.11. In C.rostrata the stomata are adaxial and are surrounded by a 'rim' of papillae produced by the subsidiary cells and the surrounding epidermal cells. As a result, the guard cells are almost completely hidden from view. In C.vesicaria the stomata are abaxial and are not surrounded by papillae, with the guard cells visible on the surface. The hybrid

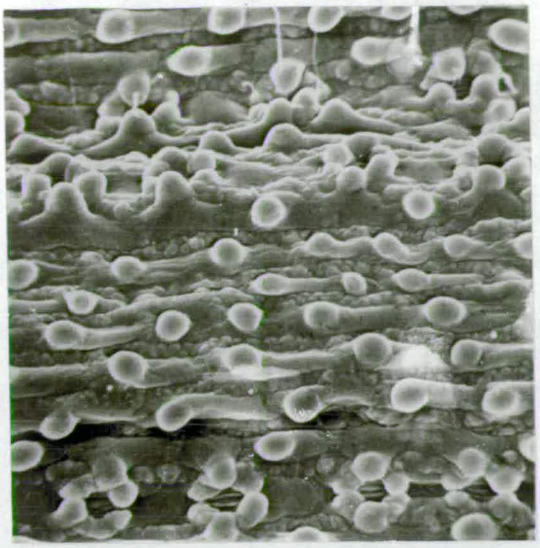
Fig.8.11 Scanning electron micrographs of adaxial leaf surfaces and stomàta.

a) C.rostrata

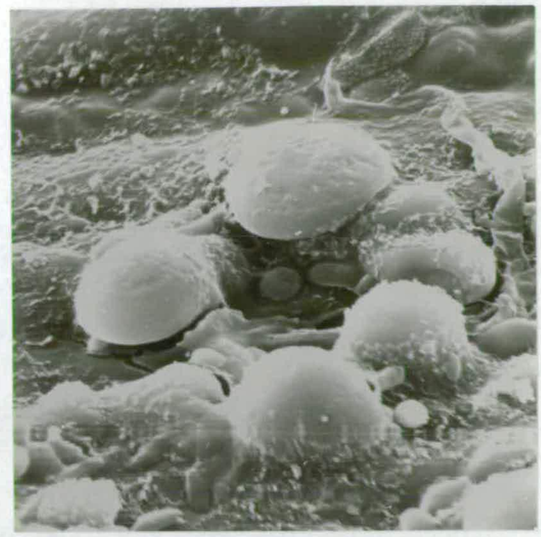
b) C.rostrata x vesicaria

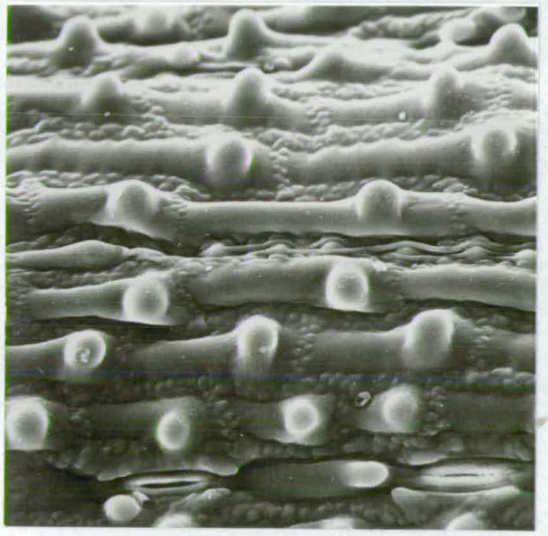
c) C.vesicaria

magnification approx x500 and x1,300



a





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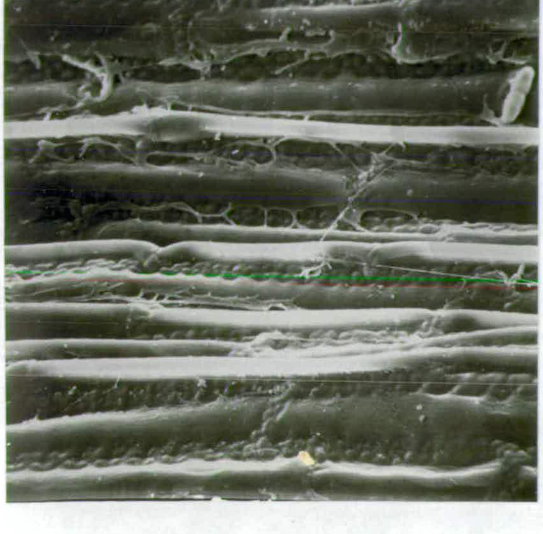
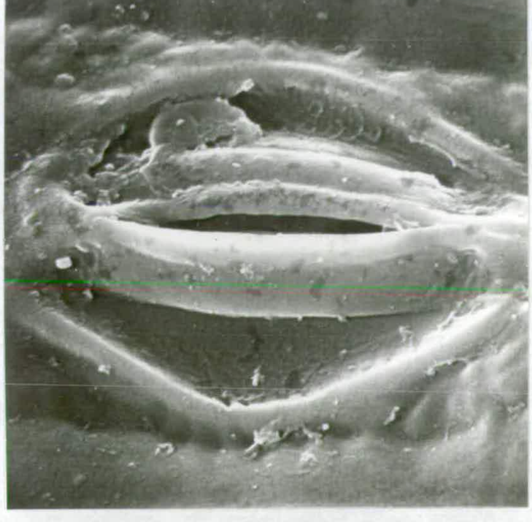


c



plants are again intermediate, with stomata scattered on both sides of the leaf. Papillae are present around the stomata but they are smaller than in C.rostrata and do not form a completely enclosing 'rim', leaving the guard cells partly open to the surface and not so strongly protected. In the hybrids papillae appear on both leaf surfaces, whereas neither parent has abaxial papillae. It is possible that the gene or gene system controlling papilla formation may be disorganised in hybrid individuals, allowing papilla formation at almost any point on the leaf surface. The production of papillae, however, appears to be closely linked with the distribution of stomata, as many of the species examined had abundant papillae only on the leaf surface which bears stomata. A hybrid producing stomata on both leaf surfaces might therefore be expected to have papillae on both. The correlation between the distributions of stomata and papillae is not absolute, however, as some species have these structures on opposite leaf surfaces, e.g. C.hirta.

The fertility data shown on Fig.8.10 were obtained by examining the seed production of the specimens measured. This is of course subject to numerous unknown environmental influences, but it seems highly significant that all the intermediate putative hybrid plants were either completely sterile or produced only a few small fruits, while the apparently typical C.vesicaria plants from the same site were fertile.

The occurrence of a large number of plants which are morphologically and anatomically intermediate between C.rostrata and C.vesicaria would suggest that some degree of hybridisation has taken place in the Grantown site. The apparent partial or complete sterility of many of the intermediate individuals supports this suggestion and would seem to be little reason to doubt the proposed hybrid origin of these plants. It is, however, difficult to estimate the extent to which hybridisation and introgression can occur. The intermediate forms in the Grantown population would appear to be F1 hybrids as they are almost exactly intermediate between the parental species. A complication is raised by the possibility that only one or two clones maybe present, so that only a single hybrid seed could conceivably have given rise to all of the intermediates in the sample. As the plants were collected from what appeared to be single tussocks, the presence of a single clone seems unlikely but this possibility cannot be completely eliminated. The only evidence suggesting the occurrence of introgression in the sense of repeated backcrossing and infiltration of genes from one species into the genotype of another (cf. Heiser, 1973), is the presence of a few individuals which possessed papillate leaf surfaces, but were fully fertile and otherwise closely resembled C.vesicaria. This is a character which is extremely rare in typical material of the latter species and its presence in a few fertile apparently typical C.vesicaria plants in the same site strongly suggest that some backcrossing has occurred, and that transfer of genes from the C.rostrata genome into C.vesicaria is possible. Again, the occurrence of a single clone representing one backcrossed seed would be compatible with the evidence available at present, but even if this is the case, the possibility of backcrossing and introgression is scarcely ruled out. As no samples of C.rostrata material were collected from this population it has not been possible to examine the degree of introgression occurring in the opposite direction. Elkington (1968), in a study of introgression in Betula, found that gene flow took place from the species with a lower chromosome number into the one with the highest number. If the same process occurred in the present case, introgression would be from C.rostrata into C.vesicaria. In Betula, however, differences of ploidy level were involved and it is doubtful if the same limitations would apply in this case where the two species differ in only 3 chromosomes in the haploid number. The cytological peculiarities of Carex (cf. Ch.6) also make it difficult to extrapolate from observations on taxa with normal chromosome behaviour. The degree of sterility shown by the intermediate plants is rather high, but this does not eliminate the possibility of fertile backcrosses. Faulkner (1972) found that although fertility was reduced in some of his hybrids of species in Sect.Acutae, high seed sets could be obtained in backcrosses with the parental species. In a population where F_1 hybrids and at least one parental species are growing intermingled, it is likely that some backcrossing will occur, even if the fertility of the hybrids is low.

Hybrids between C.rostrata and C.vesicaria appear to be fairly frequent, whenever the two parental species occur together in open, mixed habitats. Plants which appeared to be intermediate were observed in the field both at Loch Tay and at Loch Ken and the River Cree in Kirkcudbrightshire. These sites have not been investigated in detail. Herbarium material of plants resembling the hybrids in the Grantown population is available from many parts of Britain and a short list of possibly hybrid material is given in Appendix 1. Although this list is by no means complete, it indicates that in Britain at least, C.rostrata x C.vesicaria is quite widespread and not uncommon in some areas. Hybrids are also reported for much of the range over which the two parents occur, being reported for Europe (Kükenthal, 1909), Asia (Kreczetowicz, 1935) and N. America (Fernald, 1950). Kreczetowicz claims that the hybrids are very frequent and similar in appearance to C.utriculata, but they are sterile.

The total evidence available at present suggests that hybrids between C.rostrata and C.vesicaria can be produced under natural conditions and that some degree of introgression may be possible, although in many cases the hybrids are apparently sterile or of very reduced fertility. The hybrids are probably quite frequent and widespread, so that hybridisation and introgression could provide an important source of variation in both of the parental species. It is impossible at present to estimate the total extent of introgression without extensive population sampling and biosystematic studies, but if the presence of papillae on the epidermal cells of C.vesicaria is a good indicator of introgression, it would appear to be very limited since specimens showing this character are very rare. It is possible that this problem could be investigated by use of isoenzyme studies where the frequencies of different alleles may provide an indication of hybridisation. This technique has been used in studies in animals (cf. Lewontin, 1974) and Levin & Schall (1972) and Levin (1975) considered that data from seed protein composition supported the suggestion that hybridisation had occurred in some of the Phlox species which they investigated. While these techniques may provide good evidence for introgression, it would be a major undertaking to carry out a survey extensive enough to estimate the extent of introgression over a wide area.

8.4.2. Hybrids formed by other species pairs

Hybrids between members of the Vesicariae and species outside the section appear to be much less frequent than those between C.rostrata and C.vesicaria. One major problem in interpreting the situation is that in many cases it is very difficult to distinguish between odd variants of the parental species and hybrid individuals, without data on variation in the population in which the plants occurred, and the cytology of the aberrant individuals. In particular many specimens identified as C.riparia x vesicaria seem to be almost indistinguishable from the former species, and I consider that many of the reports of this hybrid are in fact only of variants of C.riparia. If the hybrid does occur, it is probably much rarer than the reports suggest. Another problem is the determination of the parentage of putative hybrids. Without supporting information it is extremely difficult to evaluate reports of Carex hybrids, even if specimens are available, and biosystematic and cytological evidence provide the only reliable guides, although even this has its limitations.

8.5 The morphology of C.grahamii

C.grahamii has always been regarded as being a somewhat enigmatic taxon and taxonomic treatments of it have been very varied (cf. Ch.2). An attempt has therefore been made to investigate the morphology of this species in some detail to compare it with C.saxatilis and C.vesicaria, the species which appear to be most closely related. In addition to the herbarium material available, population samples of C.grahamii were collected at Meall nan Tarmachan and Glen Clova to give a more detailed impression of variation within the taxon. Samples of C.saxatilis and C.vesicaria were collected in the Meall nan Tarmachan-Loch Tay area and herbarium material of Scandinavian plants has been compared with the British material. A PCA was carried out using 42 specimens of C.grahamii from population samples (21 from each site), 32 samples from other sites (herbarium material - cf. Appendix I) and 33 Scandinavian specimens. For

Fig. 8.12 Examples of utricles and glumes from C.grahamii, C.vesicaria and C.saxatilis.

a) C.vesicaria (Loch Tay)

b) C.grahamii (Meall nan Tarmachan)

c) C.grahamii (Glen Clova)

d) C.saxatilis (Meall nan Tarmachan)

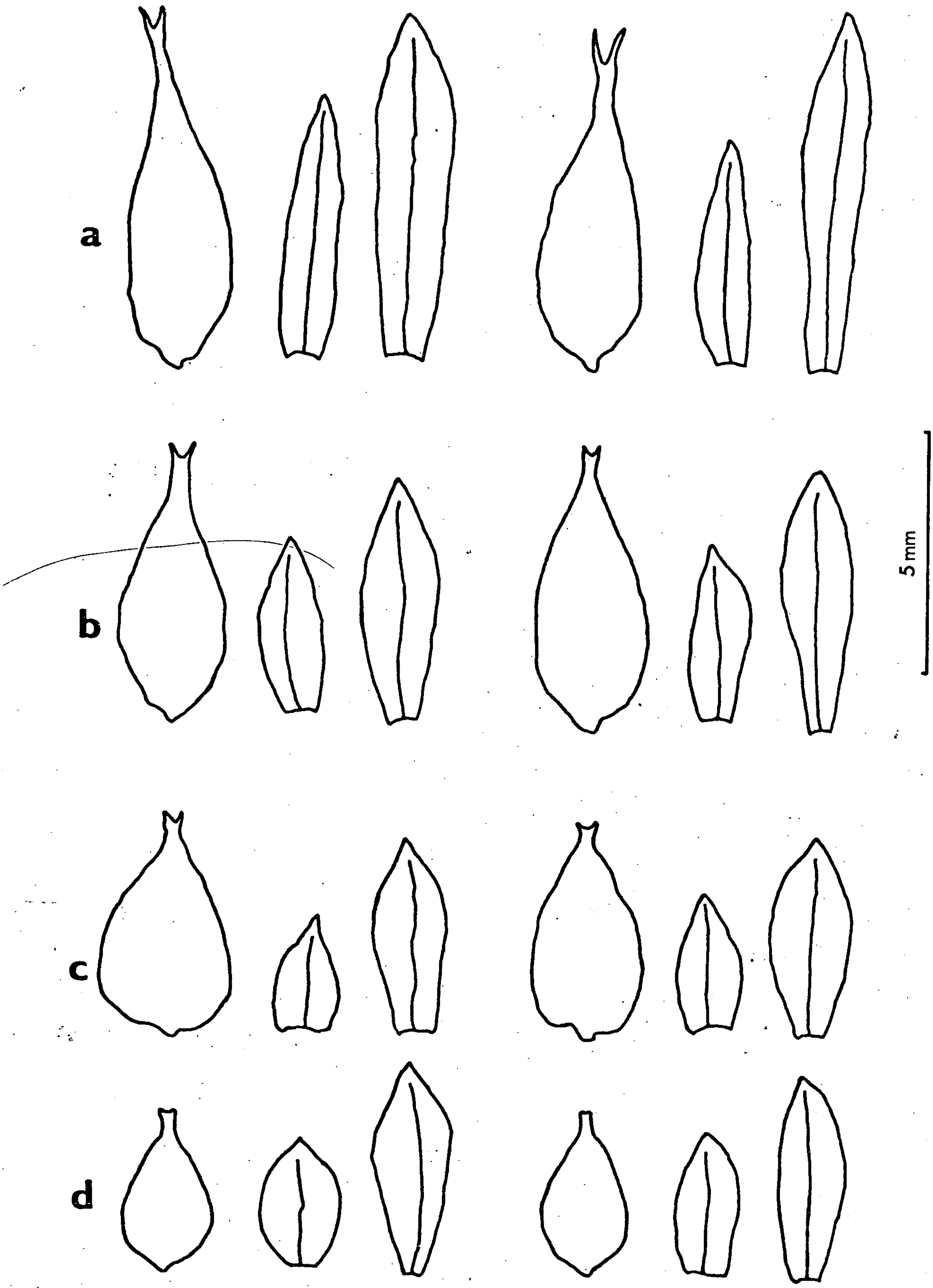
Fig. 8.13 Histograms showing distribution of utricle length and male glume length.

a) & b) utricle length

c) & d) male glume length

C.saxatilis — C.vesicaria

C.grahamii (Tarmachan) — C.grahamii (Clova)



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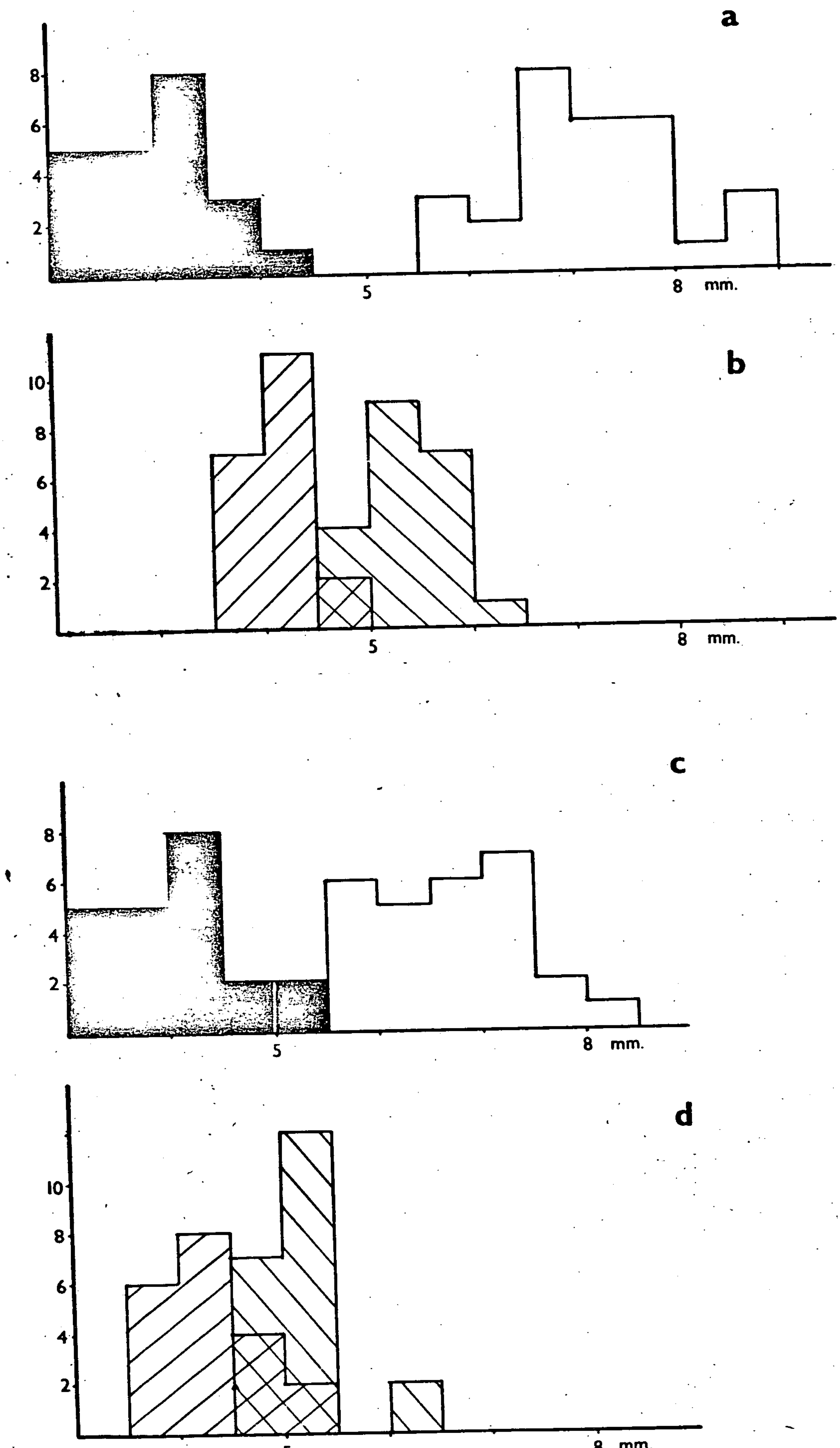


8.13

comparison, samples of C.saxatilis, C.vesicaria and C.rostrata were included along with the C.grahamii material, making a total of 220 plants in the analysis.

When measurements for plants from the Clova and Tarmachan clones were compared with those from the other species it became obvious that the plants were morphologically intermediate between C.saxatilis and C.vesicaria. In addition, however, the Clova and Tarmachan clones themselves differed, and the Tarmachan plants were generally almost exactly intermediate for almost all of the characters measured, while the Clova plants were closer to C.saxatilis. This is illustrated for utricle length and male glume in Fig.8.13, but similar differences were noted for all of the characters in which the two species differed. The eigenvectors, cumulative variance and eigenvalues for the PCA are shown in Table 8.3 and scatter diagrams for the first four components are shown in Figs.8.14-8.20. In this analysis components I and II accounted for about 75% of the total variance, representing a major part of the variation in the sample, and components III & IV account for another 10% of the variance. Components I & II alone therefore represent considerably more than the minimum of 40% of the total variance recommended by Sneath & Sokal (1974, p.304) for ordination procedures. The characters most heavily weighted in components I-IV are summarised in Table 8.4. Component I appears to primarily reflect size differences, as in the C.rostrata/utriculata analysis, since all the eigenvector elements are positive except for male and female glume widths and the latter are not so strongly weighted as the length measurements. As a result, this component separates the smaller C.saxatilis from the larger species. The second and subsequent eigenvectors are composed of a mixture of positive and negative weighting coefficients and therefore tend to reflect shape

Table 8.3 Eigenvalues, percentage variance and eigenvectors from PCA (only the first 6 eignevectors are shown).

(*C. grahamii*/*vesicaria*)

EIGENVALUES

6.78	2.14	0.77	0.58	0.51	0.35	0.27	0.20	0.18	0.11	0.09	0.04

PERCENTAGE VARIANCE

56.46	17.80	6.40	4.84	4.24	2.91	2.25	1.63	1.51	0.94	0.76	0.33

CUMULATIVE VARIANCE

56.46	74.26	80.65	85.48	89.72	92.62	94.87	96.49	98.00	98.93	99.68	100.00

EIGENVECTORS - BY ROWS

VECTOR 1	0.311	0.346	0.357	0.296	0.071	0.334	0.333	-0.238	0.308	-0.117	0.310	0.282
VECTOR 2	-0.290	-0.112	-0.133	0.342	0.559	0.075	0.118	0.188	0.229	0.453	-0.257	0.279
VECTOR 3	0.233	0.169	0.201	-0.225	-0.153	-0.113	0.119	0.613	-0.062	0.546	0.302	0.006
VECTOR 4	0.005	-0.188	-0.095	0.215	-0.579	0.139	0.349	-0.214	0.254	0.388	-0.250	-0.331
VECTOR 5	0.150	0.033	0.039	-0.118	0.033	-0.293	-0.147	-0.646	-0.289	0.504	0.049	0.309
VECTOR 6	-0.111	-0.193	-0.033	-0.098	-0.319	-0.498	0.294	0.108	0.257	-0.237	-0.058	0.605

Table 8.4 Characters most heavily weighted by PCA.

Component I.

high scores for plants with:-

Long inflorescences (0.357), bracts (0.346), utricle beak (0.334), female glume (0.333), female spike (0.311), leaf (0.310) and male glume (0.308).

Component II.

high scores for plants with:-

Wide utricle (0.559) and male glume (0.453); Long utricle (0.342)

Component III.

high scores for plants with:

Wide female glume (0.613) and male glume (0.546); Long leaf (0.302)

Component IV.

high scores for plants with:-

Narrow utricle (-0.579); Wide male glume (0.388); Long female glume (0.349); Narrow leaf (-0.331).

Note: the figure in brackets is the eigenvector element (weighting factor) corresponding to the character which it follows. The complete eigenvectors are shown in Table 8.3.

differences. Component II separates C.rostrata and C.vesicaria and these two species can be completely distinguished on this axis of the scatter diagrams (Fig. 8.14). In general, the three species produce close, well-defined clusters when plotted on components I and II, although the larger species form rather elongated clusters along component I, indicating the extensive size variation which occurs in these taxa. When the Scottish C.grahamii material is plotted on a scatter diagram using components I and II as axes, it becomes obvious that the Clova and Tarmachan populations are morphologically distinct since they form two clusters which are almost completely separated (Fig.8.15). On components III and IV (Fig. 8.17) they are also separated to a large degree, and in a 4-dimensional space formed by the first four components the two clones would form quite distinct clusters. The intermediate nature of the Tarmachan clone is evident since on axes I & II it lies almost exactly between the C.vesicaria and C.saxatilis clusters, while the Clova material forms a cluster much closer to C.saxatilis and is not directly intermediate between the two species. Components III & IV give little indication of the relationships between the C.grahamii populations and the putative parents, since the latter have very wide ranges on these axes and overlap almost completely. The area occupied by the C.grahamii samples is completely included within that of the parental species. Examples of female glume and utricle shapes for both of the C.grahamii clones are shown in Fig.8.12.

The data for herbarium material from other C.grahamii sites also show some interesting variation patterns. Material from Ben More, identified as C.grahamii was collected by Marshall, Wheldon & Wilson and others (Appendix 1). When this material is compared with the Clova and Tarmachan clones, a number of interesting points arise. The specimens collected by Wheldon and Wilson were large and obviously not typical C.saxatilis material, and this is confirmed by the PCA, since these specimens fall well outside the normal range for this species (Fig.8.19). These specimens are very similar to the Tarmachan clone on components I & II, but as can be seen from Fig. 8.20, differ in some of the shape characteristics expressed by component IV. The remaining Ben More material, however, was not readily separable from C.saxatilis although there is some overlap with the Clova clone as can be seen from the scatter diagrams. It therefore seems preferable to regard these specimens as belonging to a large form of C.saxatilis rather than C.grahamii. A further series of specimens from Meall Ghaordie was examined and again these were found to be almost indistinguishable from C.saxatilis although they are larger than the small forms which are commonest in the area.

The Scandinavian material examined presented a slightly different variation range from the Scottish forms. On components I & II, most of the specimens were located in the areas occupied by the Clova and Tarmachan clones, but there was considerable variation and some were obviously quite distinct from any of the British material examined (Fig.8.16). Some of the material overlapped with C.saxatilis, but none of the specimens seemed to closely approach C.vesicaria. On components III & IV the Scandinavian plants were much more variable than Scottish material, and although there is considerable overlap, a large number of plants fall well outside the variation range of both the Tarmachan and Clova clones (Fig. 8.18). In addition, some of the plants which resemble Scottish material on components I & II do not do so on axes II & IV and thus occupy a separate part of a multidimensional space. C.grahamii, on morphological evidence, would therefore appear to be a distinctly heterogeneous assemblage of rather variable forms.

Two major points must be borne in mind when the variation patterns of C.grahamii are considered:

1) The Clova and Tarmachan populations are samples of single clones and therefore tend to give a rather spurious impression of distinct, homogeneous groups. Morphologically distinctive clones can be detected visually in the field in C.rostrata (section 8.3.2), but if single samples from a large number of clones were considered there would be no obvious discontinuities. Between them, the variation ranges of the Tarmachan, Clova and Ben More clones encompass most of the variation exhibited by the Scandinavian samples. In addition, most of the herbarium specimens studied were representatives of separate clones, each with its own variation range, and there is no way of deciding whether or not they are extreme variants without a population sample from the clone.

2) On components III & IV at least, the variation ranges of C.saxatilis and the other 'pure' species are as wide as that of the Scottish and Scandinavian C.grahamii combined. The variation range of C.grahamii s.l. is therefore of the same order as that of each of the other species in the section.

The main conclusions to be drawn from the morphological studies of C.grahamii would seem to be that most of the material of this taxon is either directly intermediate between C.vesicaria and C.saxatilis or close to the latter species. In Scotland, the Tarmachan and Ben More clones are almost exactly intermediate, while the Glen Clova clone more closely resembles C.saxatilis. Scandinavian material is more variable, ranging from plants resembling the Tarmachan clone to

Fig.8.19 Components I & II. C.grahamii and grahamii-like forms.

(◈) Ben More (Wilson & Wheldon)

(◇) Ben More (others)

(△) Meall Ghaordie

outlines show distribution of C.saxatilis and the

Tarmachan and Clova clones of C.grahamii - cf. 8.14-8.15.

Fig.8.20 Components II & IV

symbols as in 8.19

Figs.8.14-8.20 Scatter diagrams for PCA carried out on C.grahamii, C.vesicaria, C.saxatilis & C.rostrata.

Fig.8.14 Components I & II

(○) C.vesicaria (Loch Tay)

(▣) C.saxatilis (Meall nan Tarmachan + herbarium)

(□) C.rostrata (Tamdhu)

Fig. 8.15 Components I & II

(▽) C.grahamii (Glen Clova)

(▲) C.grahamii (Meall nan Tarmachan)

outlines show distributions of the three species in 8.14

Fig. 8.16 Components I & II.

C.grahamii (Scandinavian material)

outlines show distributions of Scottish C.grahamii clones - Fig. 8.15.

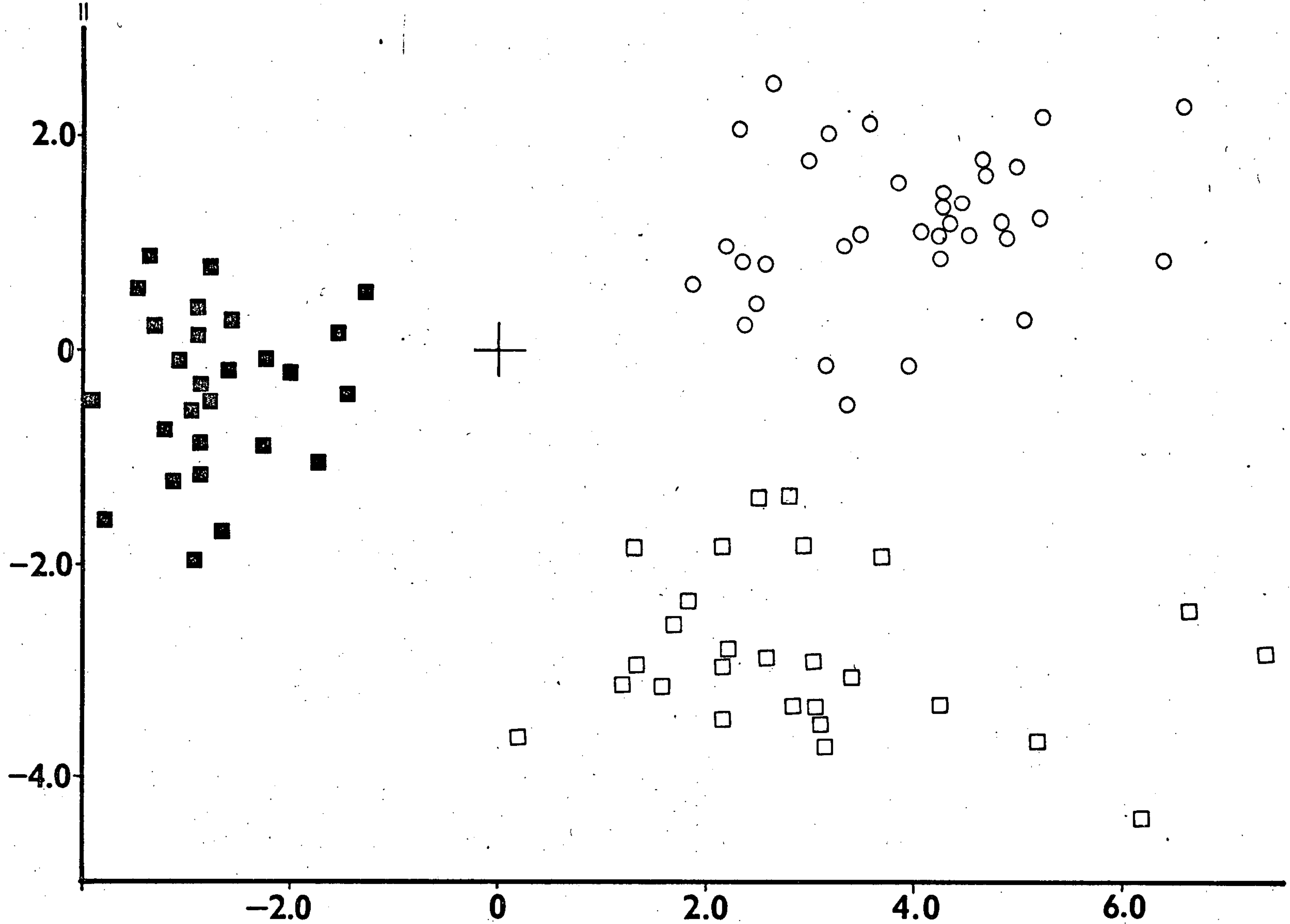
Fig.8.17 Components III & IV

C.grahamii

symbols as in 8.15

Fig.8.18 Components III & IV

C.grahamii (Scandinavian material)



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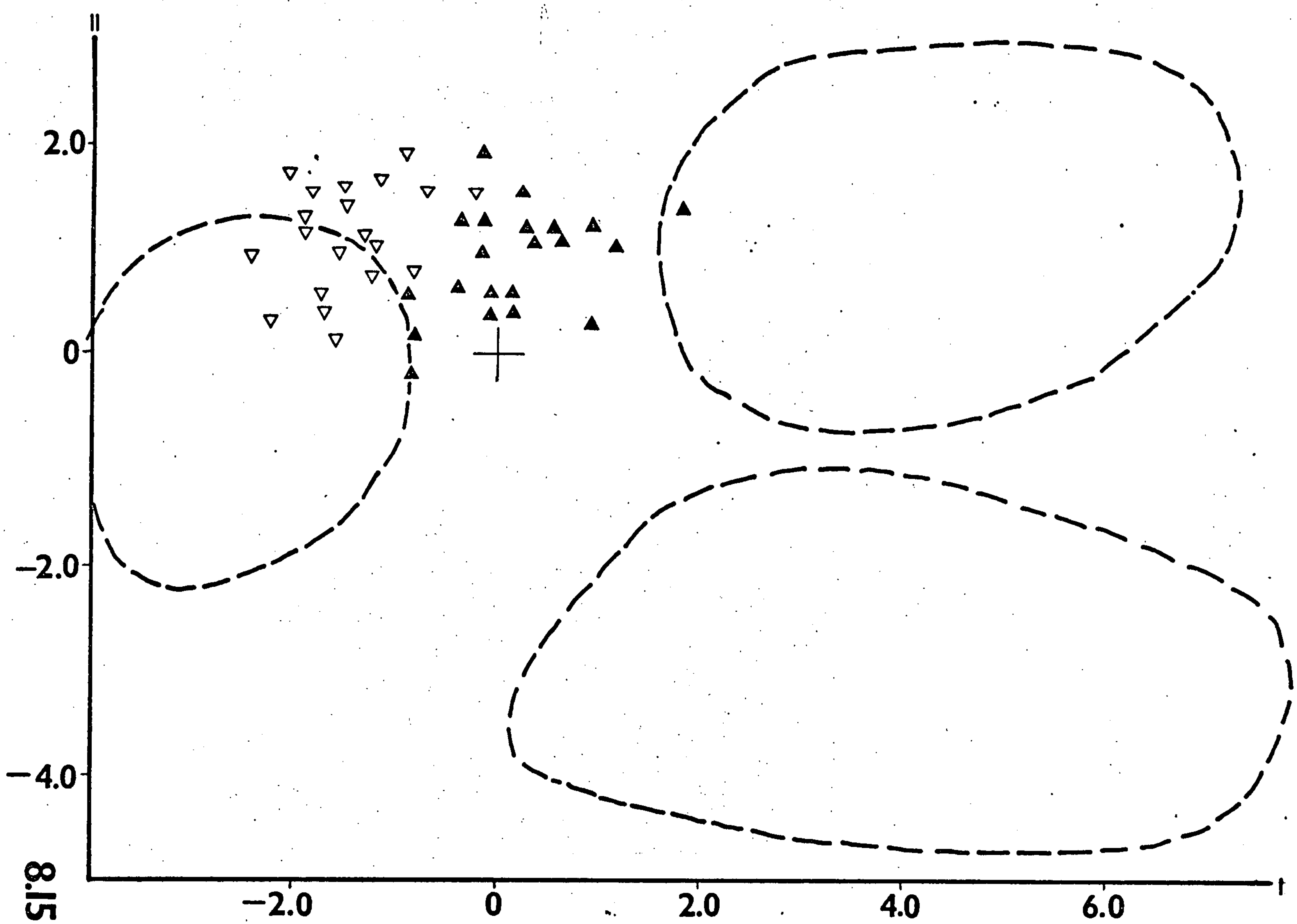

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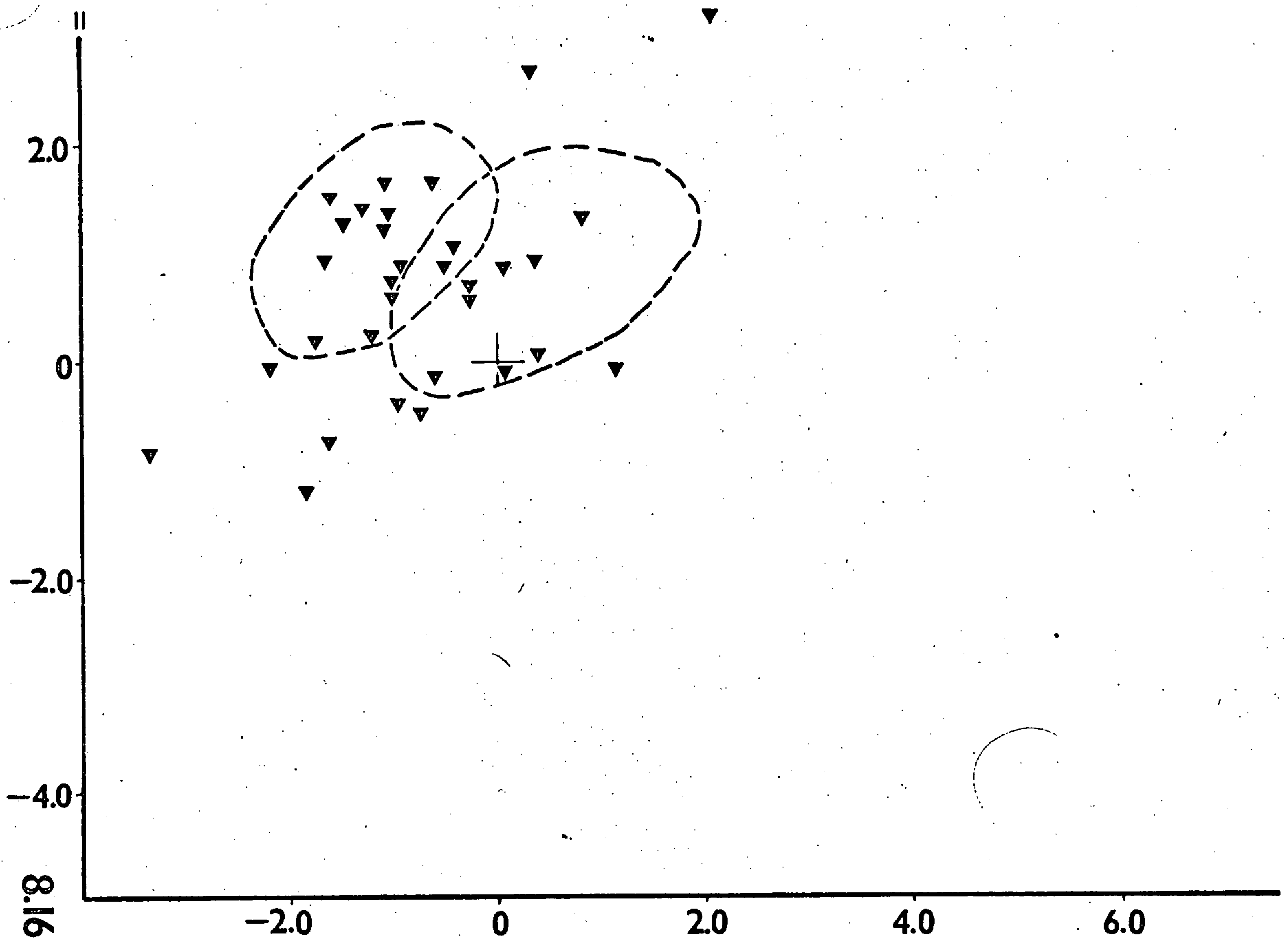

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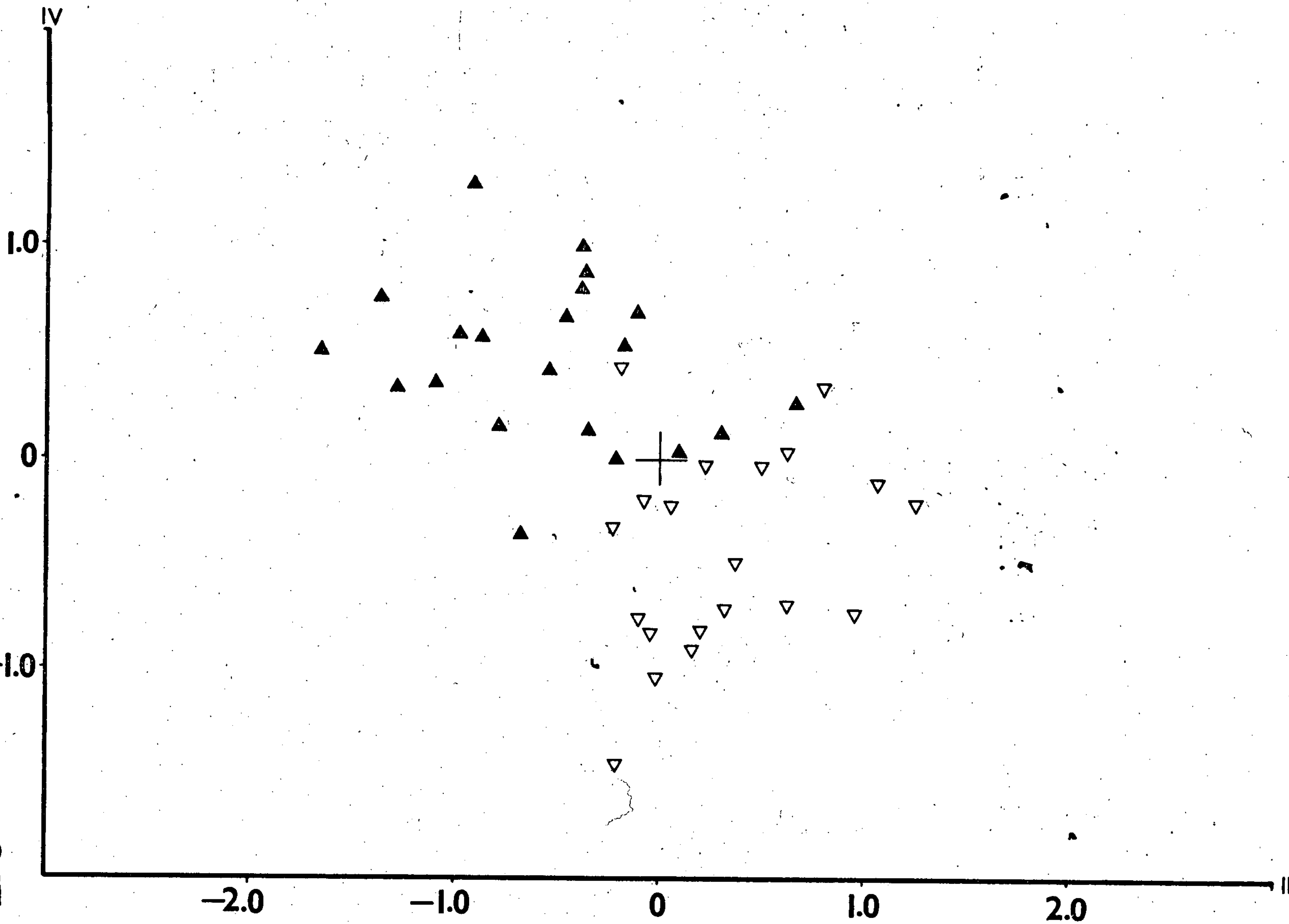

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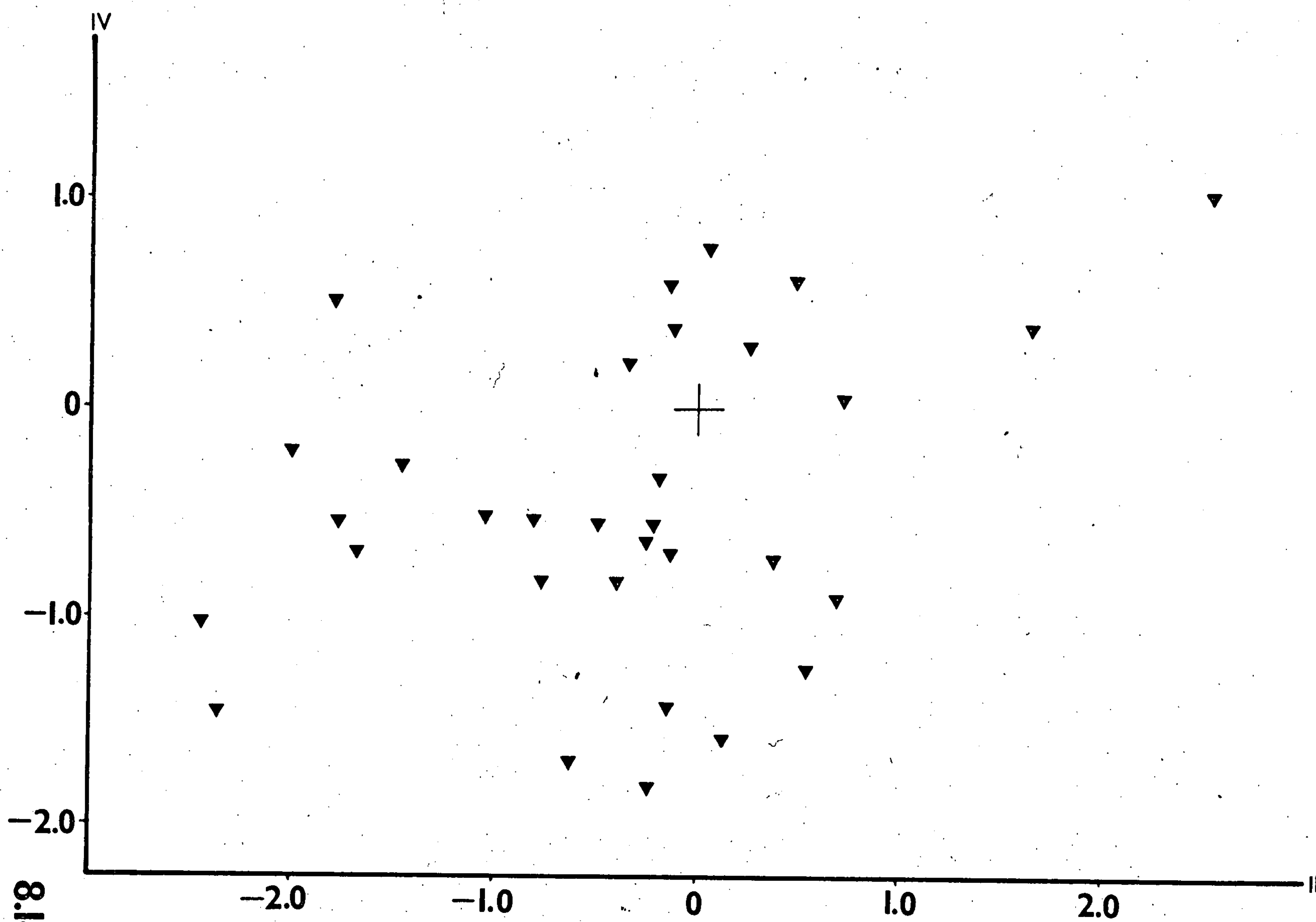

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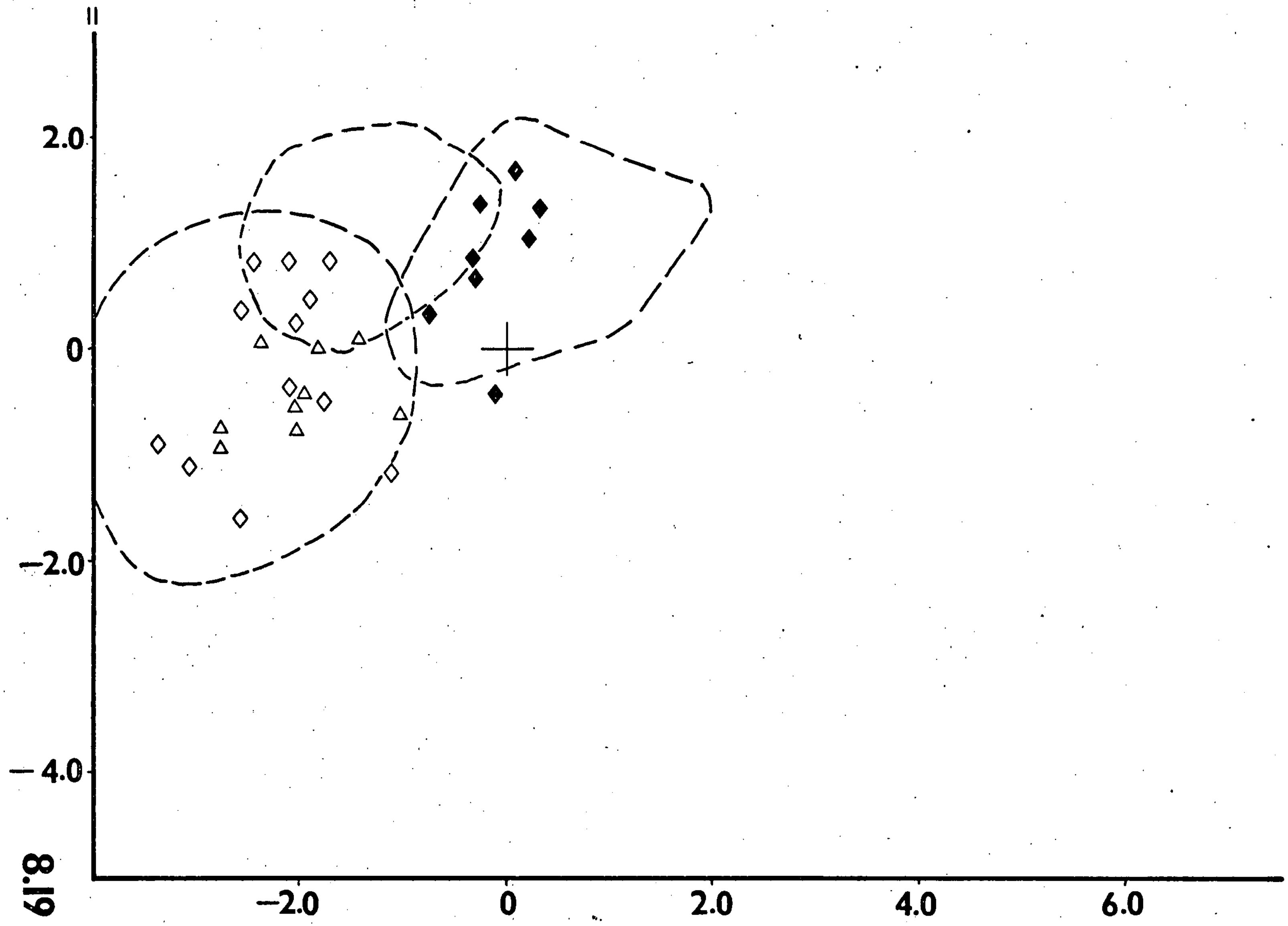
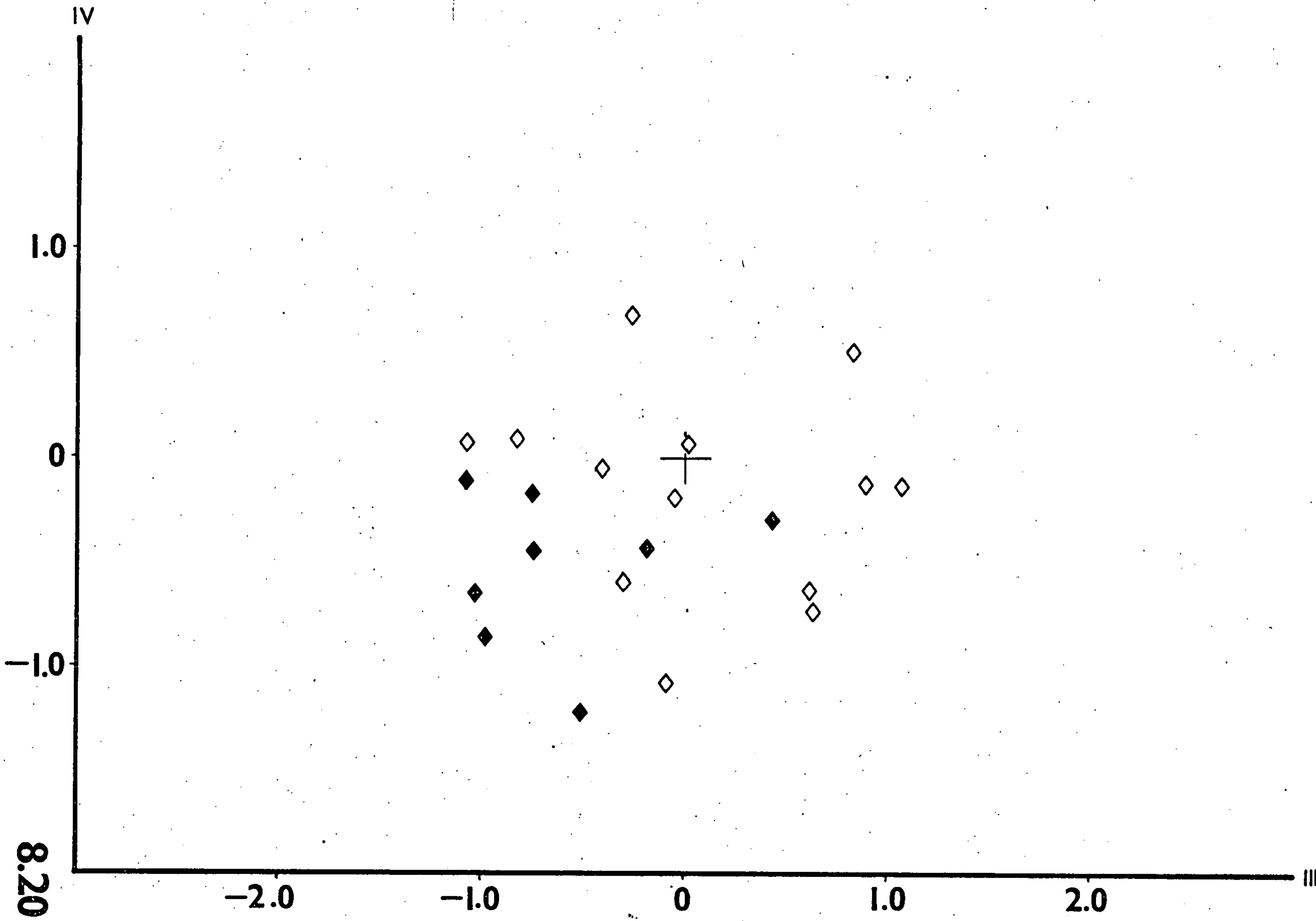


8.19



8.20

plants which are more or less indistinguishable from C.saxatilis. There would appear to be considerable justification, in morphological terms, for the view that C.grahamii is a hybrid between C.vesicaria and C.saxatilis, together with a series of backcrosses linking it with the latter species. The Glen Clova clone would seem to represent a possible backcross since it is much closer to C.saxatilis, and the presence of numerous large forms of this species which approach the Clova material in size and shape may indicate that some extensive backcrossing and introgression have occurred. It is, however, very difficult to distinguish between the effects of introgression and normal size variation in C.saxatilis and it is therefore impossible at present to estimate the importance of introgression from C.vesicaria as a source of variation in C.saxatilis. There is no evidence from the present study to suggest that C.rostrata has been involved in the ancestry of C.grahamii, although from an ecological viewpoint, this species seems more likely than C.vesicaria. This species is quite distinct morphologically and there were no plants, British or Scandinavian, of C.grahamii which were intermediate between C.saxatilis and C.rostrata. This hybrid is, however, reported from Scandinavia.

8.6 Variation in C.vesicaria and C.saxatilis.

Variation patterns in these species have not been so closely examined as in C.rostrata, but it is readily apparent that similar widespread variability occurs. The extent of interclonal variation has not been investigated, and it is much more difficult to delimit clones by morphological characters in these two species since C.vesicaria often shows a more clumped, almost tussocky habit, rather than the uniform stands characteristic of C.rostrata, while

C.saxatilis shows a much more open growth form with widely scattered single stems or small clumps of stems. It is not clear at present how extensive single clones may be in these species. C.vesicaria does not show such a wide range of variation on a local scale, but when material from the whole of Europe is considered, a number of variations can be seen. Many Scandinavian and N.European forms have shorter, wider utricles than typical British material. This difference can be seen when the illustrations for Scandinavian material in Lid (1963) and British material in Jermy & Tutin (1968) are compared. It is unlikely that any formal recognition of separate taxa is required in this case, but the possibility that a series of races of different origins may occur in Europe cannot be ruled out. When N.American and Asiatic material is considered, the situation becomes much more complex, with numerous varieties and species recognised by different authors (e.g. Kreczetowicz, 1935; Fernald, 1950), but it is very difficult to find clear demarcation lines between the taxa in the complex. It is interesting that differences in chromosome number, similar to those in C.rostrata, are reported, but in this case, the lower number (2n = 74) is found in N.American and Asiatic plants while the higher number (2n = 82) is found in Europe. It remains to be seen whether, as in C.rostrata, these differences are correlated with morphological variation.

C.saxatilis is also extremely variable. Much of the material found in Britain and N.Europe consists of small plants with short, erect, rounded, more or less sessile female spikes, but variants with much longer, often pedunculate and sometimes drooping female spikes are frequent. A large number of formal names have been proposed, mainly at varietal level, and it is extremely difficult to disentangle the complex nomenclature and taxonomy of these forms. The most commonly applied name for the pedunculate form appears to be var. alpigena Fries. Hulten (1962) divides the species into C.saxatilis s.s. and C.saxatilis ssp. laxa Kelela, considering that most of the Asiatic and N.American material belong to the latter. He also includes C.physocarpa Presl. in ssp.laxa but this may be an over-simplification of the problems within this complex since reports of chromosome numbers of 2n = 60 have been made for that species. If Hulten is correct in his view, then at least two different ploidy levels appear to be present within C.saxatilis s.l. (2n = 60,80) and if C.miliaris is included as Fernald (1950) suggests, the count of 2n = 40 reported for this species (cf. Ch.6) leads to further complications in the cytology of the group. It seems likely that several species are present in the complex, but it may prove extremely difficult to find suitable characters to diagnose them unambiguously. The amount of material studied in the present survey is insufficient to enable any definite conclusions to be reached on variation patterns in C.vesicaria and C.saxatilis : indeed the only fact which emerges clearly is that a thorough revision of all the species in Sect.Vesicariae is badly needed before any further progress can be made in understanding the evolutionary development of the group. This would require a detailed study of the complex in N.America, the Bering Straits area and E.Asia since these appear to be critical centres of extensive morphological and cytological diversity. Morphological studies would have to be supported by biosystematic and possibly chemotaxonomic investigations if any fundamental understanding of the significance of the variation patterns of these species is to be achieved.

8.7 Conclusions

In *C.rostrata* environmental factors such as water depth appear to promote considerable variation in size of the plants, but their effect on shape characteristics seems to be much more limited. It is possible to recognise morphologically distinct clones from different parts of the habitat on the basis of their general appearance, even when there is widespread size variation within each clone. Inter-clonal variation has a much stronger effect on the shape characters which influence the form of the utricles and the female spike and is probably responsible for a large part of the small scale local variation found in this species. The population structure in *C.rostrata* shows many similarities to that of *Phragmites communis*, in that single clones may spread vigorously by vegetative means and cover a large area, effectively forming a single individual with a long lifespan (? 50 years or more). Variation on a large scale geographical basis is also very important and has produced an exceedingly complex series of intergrading forms in all three of the widespread species in the section. On morphological grounds, it would appear that the taxonomic rank of the N.American form currently referred to as *C.rostrata* var.*utriculata* should be raised to subspecific or specific level. The situation in *C.vesicaria* and *C.saxatilis* is more confused and requires a detailed study.

Variation caused by hybridisation and introgression appears to be relatively common in *C.vesicaria* and *C.rostrata* at least, but the full extent of introgression is at present unknown. The evidence available would suggest that hybridisation has a very local effect, and that introgression is probably not a highly significant source of variation, although hybridisation between these two species is quite frequent over almost all the areas in which both occur. Hybridisation between other species pairs is probably less frequent and further investigation is needed to establish the status of many of the putative hybrids because of the strong possibility of misidentification.

C.grahamii, so far as can be determined from morphological evidence, consists of a series of F_1 hybrids and backcrosses between C.saxatilis and C.vesicaria. It seems most unlikely that any other species are involved, even in the more variable Scandinavian material. Morphologically at least, the use of the name C. x grahamii (= C.saxatilis x vesicaria) appears to be justified.

CHAPTER 9 DISCUSSION AND CONCLUSIONS

9.1 Introduction

This chapter is intended to review briefly the results obtained from different parts of this investigation, and to assess the extent to which these have provided explanations for the problems under study. The major problems outlined in Chapter 1 were:

1) In view of the apparently widespread hybridisation between members of Sect. *Vesicariae* and a number of sections in subgenus *Carex*, should sectional limits be extended to include all the members of the hybridising complex in a single section, as suggested by some authors, e.g. Kreczetowicz (1935) and Koyama (1962)?

2) Many members of the *Vesicariae* are extremely variable. What are the sources of this variation and what is its taxonomic significance?

3) What are the affinities between the species of Sect. *Vesicariae*, and in particular, *C. grahamii*? What is *C. grahamii*?

9.2 Sectional limits in the Vesicariae complex.

The main question in this case is whether or not the sections which appeared to be linked by hybridisation (referred to as the '*Vesicariae* complex' in the following discussion) should be united as a single section. In the extreme case, this proposal would entail merging Sects. *Vesicariae*, *Pseudocypereae*, *Hirtae* and *Paludosae* in one large heterogeneous section. Sects. *Lupulinae* and *Tentaculatae* would also be involved but these have not been investigated in detail. The evidence for and against the extension of Sect. *Vesicariae* is outlined below, along with the geographical distributions of the sections involved (Figs. 9.2-9.7).

9.2.1 Seed protein composition

The evidence available at present from this source is unfortunately very fragmentary (Chapter 4). Serological techniques (Section 4.3) were not very successful in _Carex_ species because of low protein contents and apparent low antigenicity of _Carex_ seed proteins. Gel electrophoresis (Section 4.4) was much more successful, but the results obtained so far are too limited to permit any definite conclusions to be reached on sectional limits. It would appear that many species from different sections have distinctive seed protein complements, but it is not yet known if the protein banding patterns observed are homogeneous within the sections which the species belong to. Two members of Sect. _Vesicariae_ - _C.vesicaria_ and _C.saxatilis_ - had very similar protein compositions, while _C.rostrata_ appears to be more or less distinct and shows a rather different banding pattern. _C.pseudocyperus_ from Sect. _Pseudocypereae_ shows strong similarities to _C.vesicaria_ and _C.saxatilis_, but other members of the _Vesicariae_ complex have not been tested.

9.2.2 _Anatomy_

Assessment of anatomical evidence (Chapter 5 - sections 5.4-5.5) is difficult for two main reasons -

1) The proportion of species surveyed at present is still rather low and it is possible that further studies will radically alter the interpretation given here.

2) There is considerable variation within sections and many of the characters which appear to differentiate between sections are somewhat vague and difficult to define. Anatomy cannot therefore be used with any degree of certainty as a guide to sectional classification, but the results obtained would seem to indicate that most of the sections in the _Vesicariae_ complex show distinctive combinations of anatomical characters. I find it difficult to agree completely with

Mazel (1891) and Akiyama (1942) who decided that anatomical characters are of no value in classification above the species level. There is some agreement between anatomy and morphological subdivisions, though it must be admitted that it is weak and insufficient to provide a good basis for sectional delimitation without strong supporting evidence. Sects.Vesicariae and Paludosae appear to possess a number of characters which consistently distinguish between the two sections and also separate them from the remainder of the complex, while though the Hirtae are more heterogeneous, all the species investigated were distinct from the Vesicariae. Similarly, C.pseudocyperus differs from the other sections, but the significance of the differences noted is not known because only this species has been investigated in Sect.Pseudocypereae.Sect.Lupulinae appears to be quite close to the Vesicariae on anatomical grounds, supporting the close morphological relationship suggested by Kükenthal when he made both of these taxa subsections of his Sect. Physocarpae, but only two species have been examined. Sect.Tentaculatae has not been investigated.

Bearing in mind the limitations outlined above, anatomical evidence would seem to support the present system of delimitation of the complex into a series of separate sections, rather than a single large section, but it must be emphasized that the distinctions between sections are not clear-cut and anatomical data are undoubtedly of greatest value at species level. There is little evidence from anatomy to support larger scale grouping of sections, e.g. at subgeneric level.

9.2.3. Cytology

The cytological evidence obtained in the present survey (Chapter 6 - Section 6.3) and reported in published accounts (Chapter 6 - Section 6.5) suggests that groupings of sections within the Vesicariae complex can be made on the basis of chromosome number, since each section tends to have a comparatively narrow range of numbers. Although occasional counts outside the main range occur, Sects. Vesicariae and Paludosae have higher chromosome numbers than any of the other sections. Sect. Lupulinae tends to have much lower numbers, while Sects. Tentaculatae and Pseudocypereae are intermediate and the Hirtae differ markedly from all the other sections in the complex in possessing a very wide range of numbers. Although the use of such data is subject to considerable uncertainty, it would appear that cytological evidence is compatible with a division of the Vesicariae complex into at least four groups.

9.2.4 Biosystematics.

The evidence so far available from crossing experiments suggests that while all the British species of Sect. Vesicariae are able to form viable F_1 hybrids, there is a significant barrier to gene flow between members of this section and Sects. Hirtae and Pseudocypereae. Crosses with Sect. Paludosae have not been carried out. A crossability barrier also seems to exist between Sects. Hirtae and Pseudocypereae, indicating that each of the three sections tested forms a genetically isolated group in which gene flow from other species must be extremely rare or non-existent. This suggests strongly that the present sectional delimitation of the Vesicariae complex ought to be maintained.

9.2.5 Geographical distribution

The geographical distributions of the sections of the Vesicariae complex demonstrates a number of interesting differences. These are shown in figs. 9.2-9.7 and the method of map construction is explained in Fig. 9.1. An approximate isoflor map can be produced by combining the areas of different species as shown in the diagram. Although this technique is probably less accurate than the grid system described by Davis & Heywood (1963), the latter is much more time-consuming to construct and will only be more accurate if a fairly small grid size is used. This method also demands accurate knowledge of the distribution ranges of the species being mapped - information which is not available for many of the species included in the present survey. This technique is very similar to that used by Kupicha (1974). The individual species distributions are based on the maps produced by Hulten (1958, 1962) and Meusel *et al.* (1965) and where no maps were available, an approximate distribution area was plotted out using data given by Kükenthal. It must be stressed that these maps are intended only as an approximate guide to the major features of distribution of the sections and do not provide very accurate details of individual species distributions. The delimitation of the sections follows Kükenthal except for the separation of the *Lupulinae* and *Tentaculatae* as sections. Where segregate species not recognised by Kükenthal have been mapped by Hulten and Meusel *et al.*, these have been included so that the total number of species mapped is generally greater than that recognised by Kükenthal.

The main features of the distribution patterns can be summarised as follows:

1) Sect. *Vesicariae* shows a strongly circum-arctic to circum-north temperate distribution, with the highest concentration of species occurring in Scandinavia, Eastern Asia and Eastern North America. None of the species in this section extends further south than the tropic of Cancer, and there are no major disjunctions in the range of the section.

2) Sect. *Pseudocypereae* has a much more disjunct distribution. The main centres of diversity appear to be in Eastern North America

and in Southern South America. The range of this section in both N. America and Eurasia is more southern than that of the Vesicariae and the Eurasian part is split into two separate areas - Europe with Western and Central Siberia, and Korea/Japan. Species considered to belong to this section occur also in South Africa, Madagascar and Australasia, including three species in New Zealand.

3) Sect. Paludosae also has a disjunct distribution. This group has a centre of diversity in the area stretching from Northern China through Korea to Japan. The Eurasian range is continuous but more southern than in the Vesicariae. Other species are present in Eastern North America, South America, South Africa and Australia/New Zealand. Two species, C.pumila and C.trifida, are common to both Eastern Australia/New Zealand and South America and the former is also found in E.Asia.

4) Sect. Hirtae also has a disjunct distribution with three main areas: Eurasia, with a continuous range from Europe to Eastern Asia and extending south to the Himalayas; North America, extending from Alaska to the east coast (Eastern North America appears to be the main centre of diversity); South America, mainly along the Andes. No representatives of this section occur in Africa or in Australasia.

5) Sect. Lupulinae differs from the preceding sections in having a very restricted distribution in the Eurasian part of its range, being found only in extreme Eastern Asia. The main centre of diversity is in Eastern North America, and there are two South American species in this section.

6) Sect. Tentaculatae has a similar distribution to the previous section, but there is only one species in Eastern Asia and there are representatives of the section in Western North America and Mexico

Fig.9.4 Sect.Paludosae

species included:

C.riparia	C.acutiformis	C.hyalinolepis
C.rugulosa	C.lacustris	C.chilensis
C.anisoneura	C.braxilensis	C.trifidus
C.glaucescens	C.clavata	C.argyii
C.pseudochinensis	C.scabrifolia	C.nutans
C.platyrhyncha	C.heterostachya	

Fig.9.5 Sect.Hirtae

species included:

C.lasiocarpa	C.lanuginosa	C.fedia
C.drymophila	C.atherodes	C.aematorrhyncha
C.beechyana	C.striata	C.tweediana
C.ligulata	C.vestita	C.hirta

Fig.9.6 Sect.Lupulinae

species included:

C.macrosolen	C.intumescens	C.dusenii
C.grayii	C.subulata	C.idzuroei
C.michauxiana	C.halei	C.lupulina
C.folliculata	C.grandis	

Fig.9.7 Sect.Tentaculatae

species included:

C.dickinsii	C.lurida	C.retrorsa
C.stenolepis	C.tuckermanii	C.squarrosa
C.bullata		

Fig.9.1 Construction of isoflor maps.

a) individual species areas plotted

b) areas combined to give isoflors

Fig.9.2-9.7 Distribution maps for Sections in the _Vesicariae_ complex.

Fig.9.2 Sect._Vesicariae_.

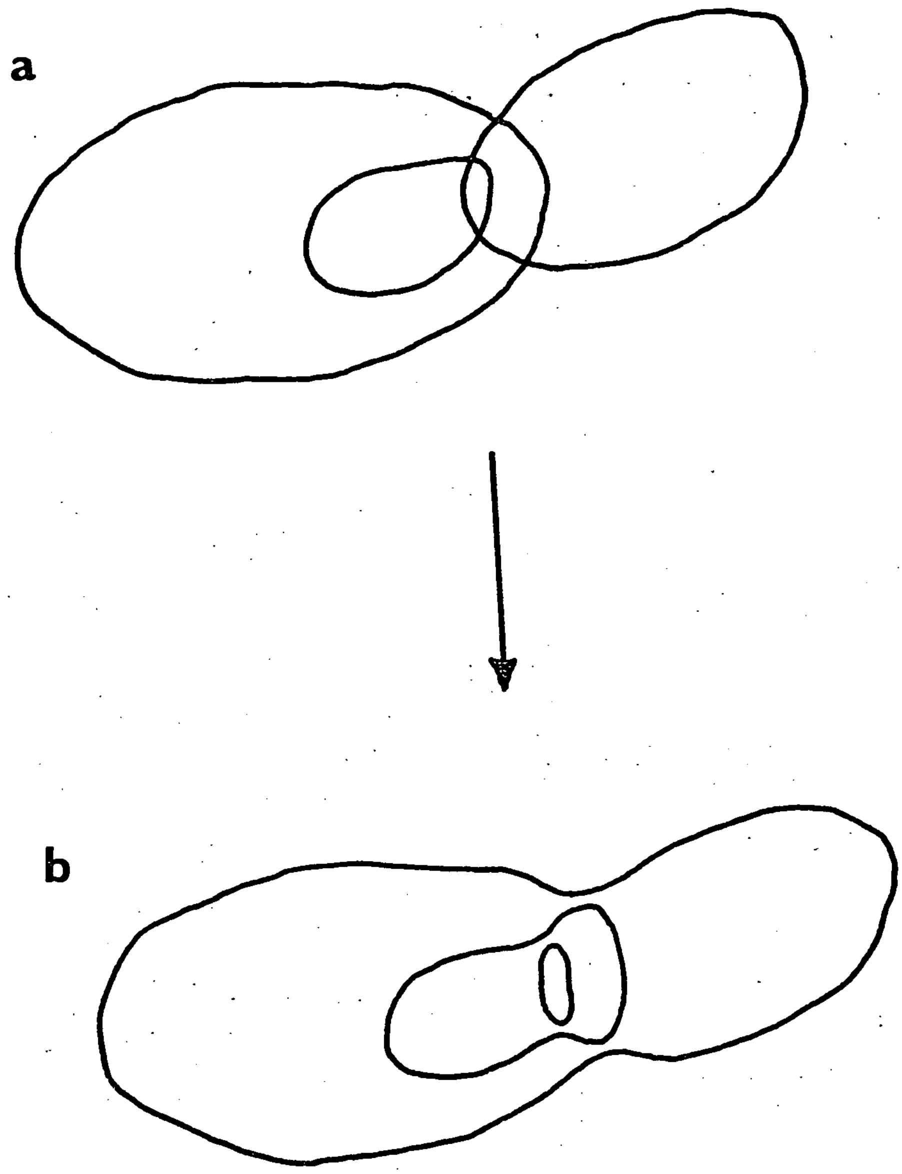
species included:-

C.rostrata	_C.vesicaria_	_C.saxatilis_
C.rhyncophysa	_C.miliaris_	_C.obscuriceps_
C.rotundata		

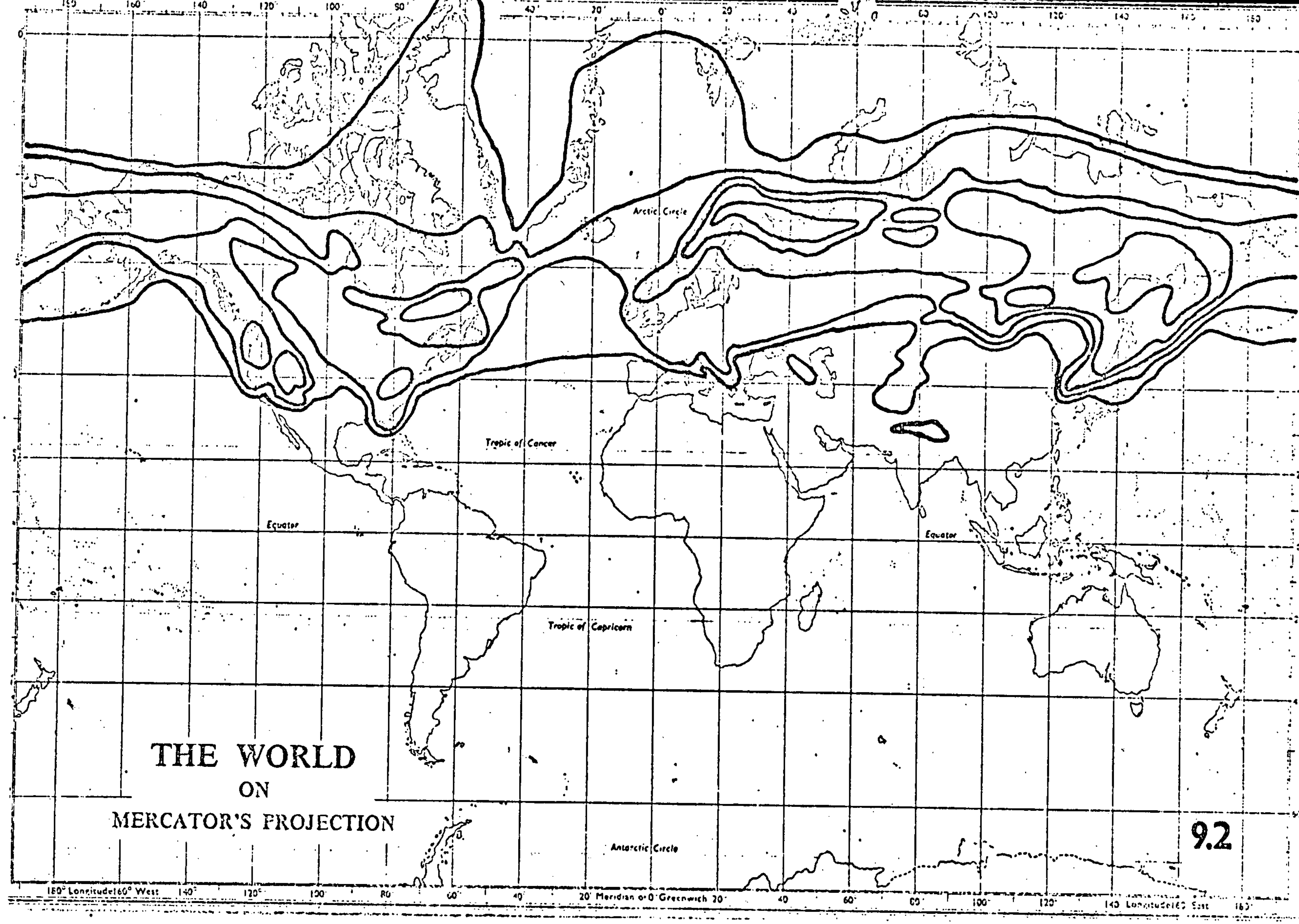
Fig.9.3 Sect._Pseudocypereae_

species included:

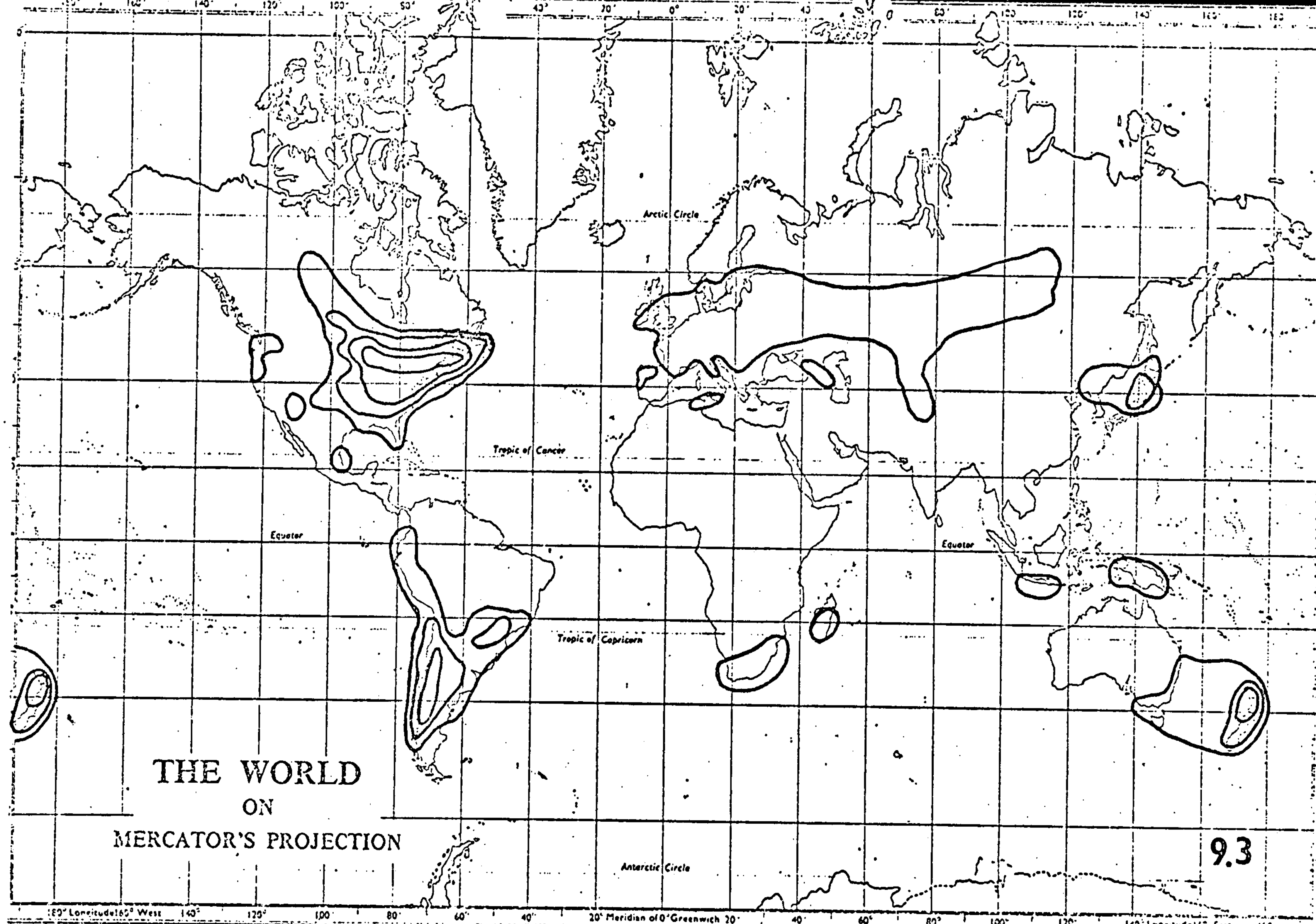
C.pseudocyperus	_C.fascicularis_	_C.comosa_
C.polysticha	_C.hankeana_	_C.cognata_
C.forsterii	_C.vacillans_	_C.sphaerogyna_
C.capricornis	_C.acutata_	_C.hystricina_
C.schweinitzii	_C.arizonensis_	_C.purpurovaginata_

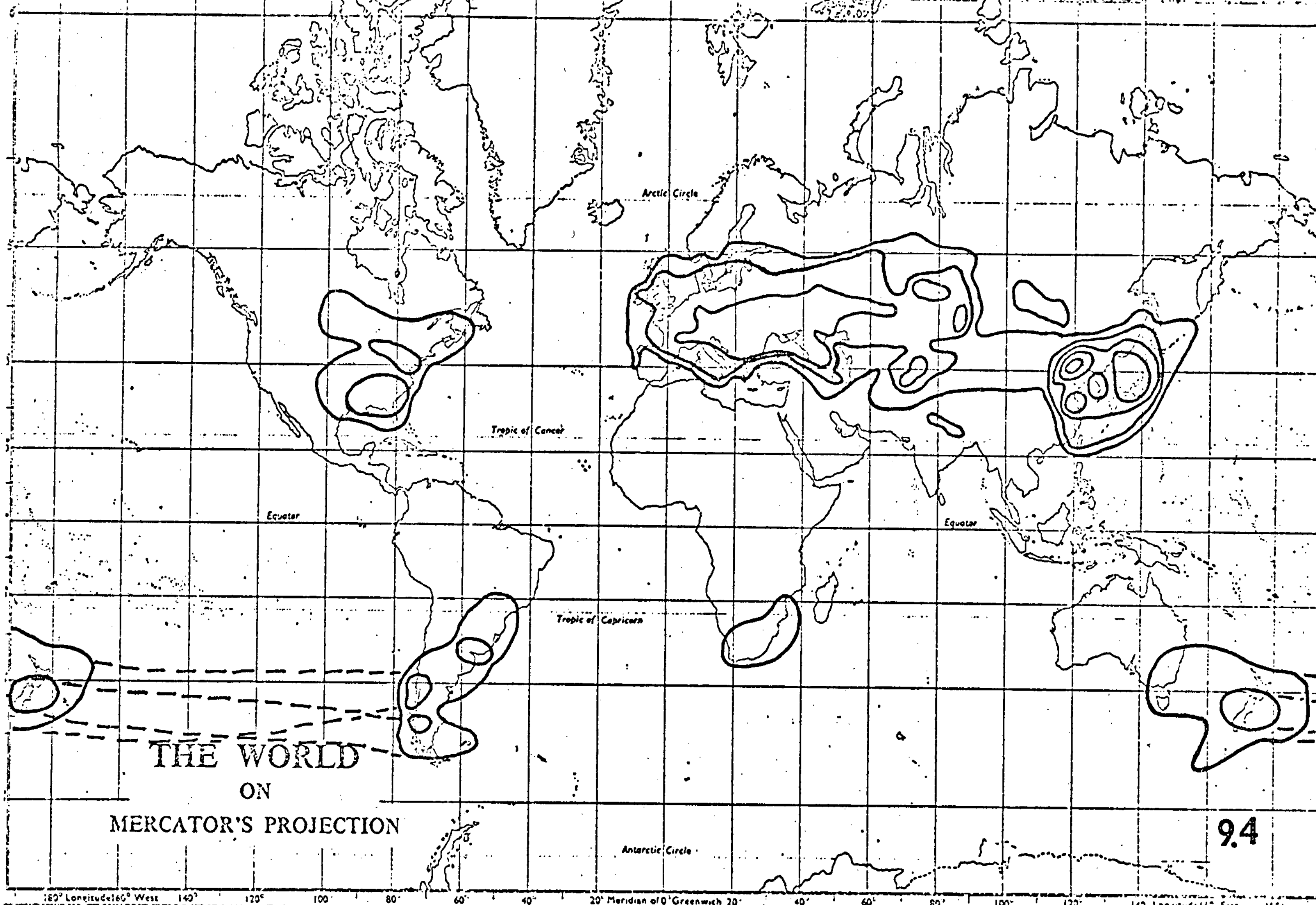


9.1

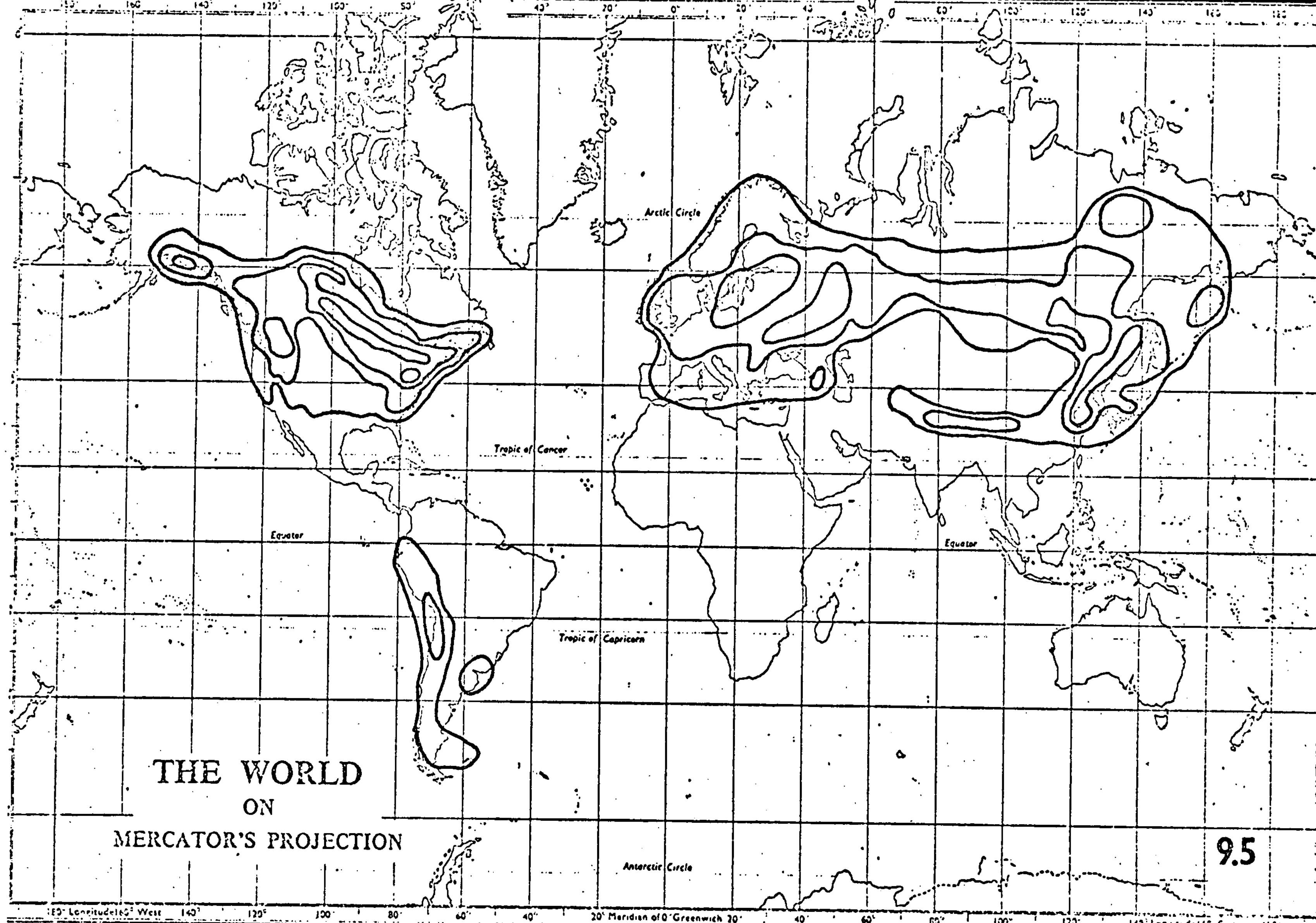


THE WORLD
ON
MERCATOR'S PROJECTION
9.2
Arctic Circle
Tropic of Cancer
Equator
Equator
Tropic of Capricorn
Antarctic Circle
180° Longitude 160° West
Meridian of Greenwich
Longitude 160 East


THE WORLD
ON
MERCATOR'S PROJECTION
Arctic Circle
Tropic of Cancer
Equator
Equator
Tropic of Capricorn
Antarctic Circle
9.3


Arctic Circle
Tropic of Cancer
Equator
Equator
Tropic of Capricorn
Antarctic Circle
THE WORLD
ON
MERCATOR'S PROJECTION
20° Meridian of 0° Greenwich 20°

9.4


THE WORLD
ON
MERCATOR'S PROJECTION
9.5
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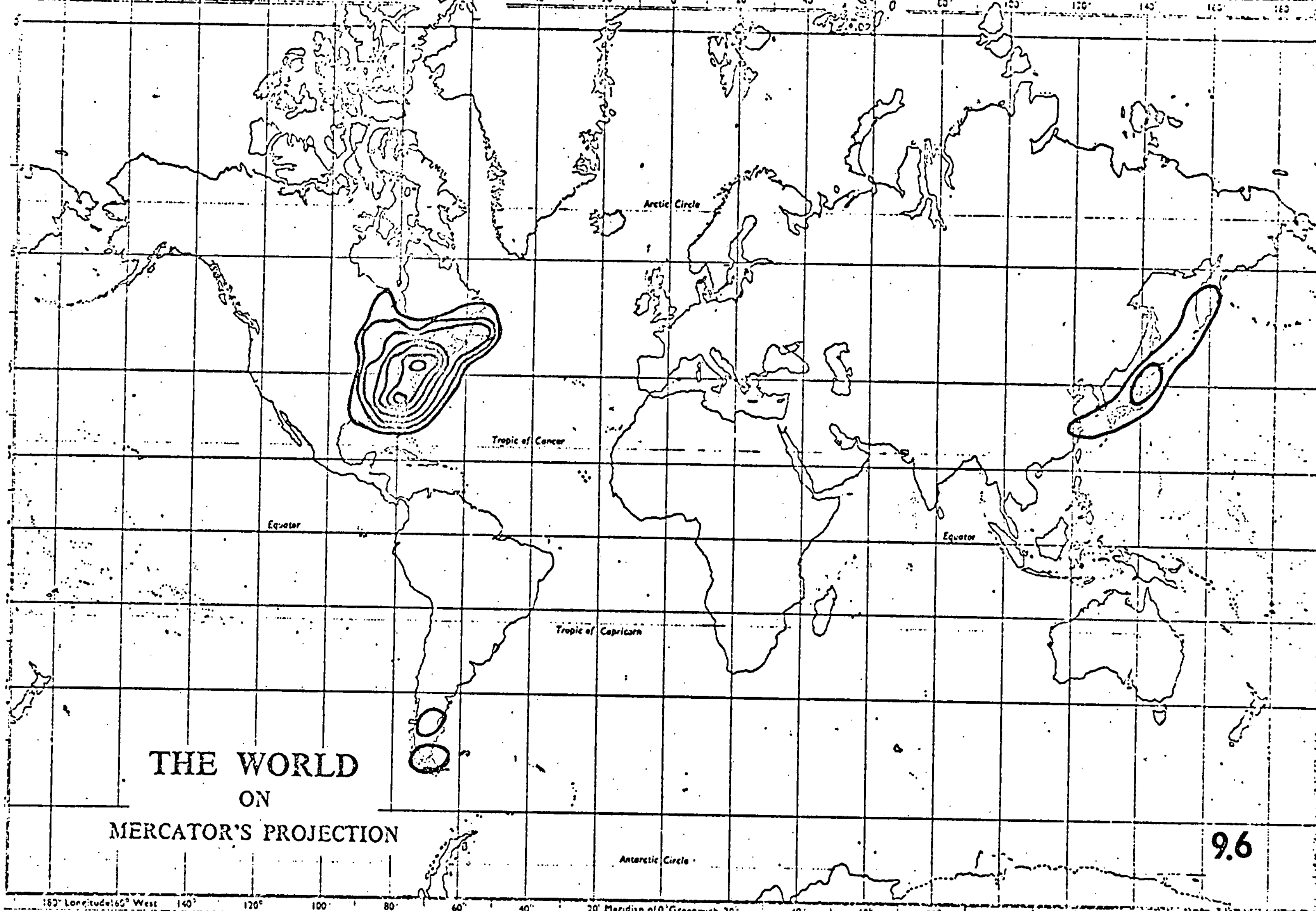
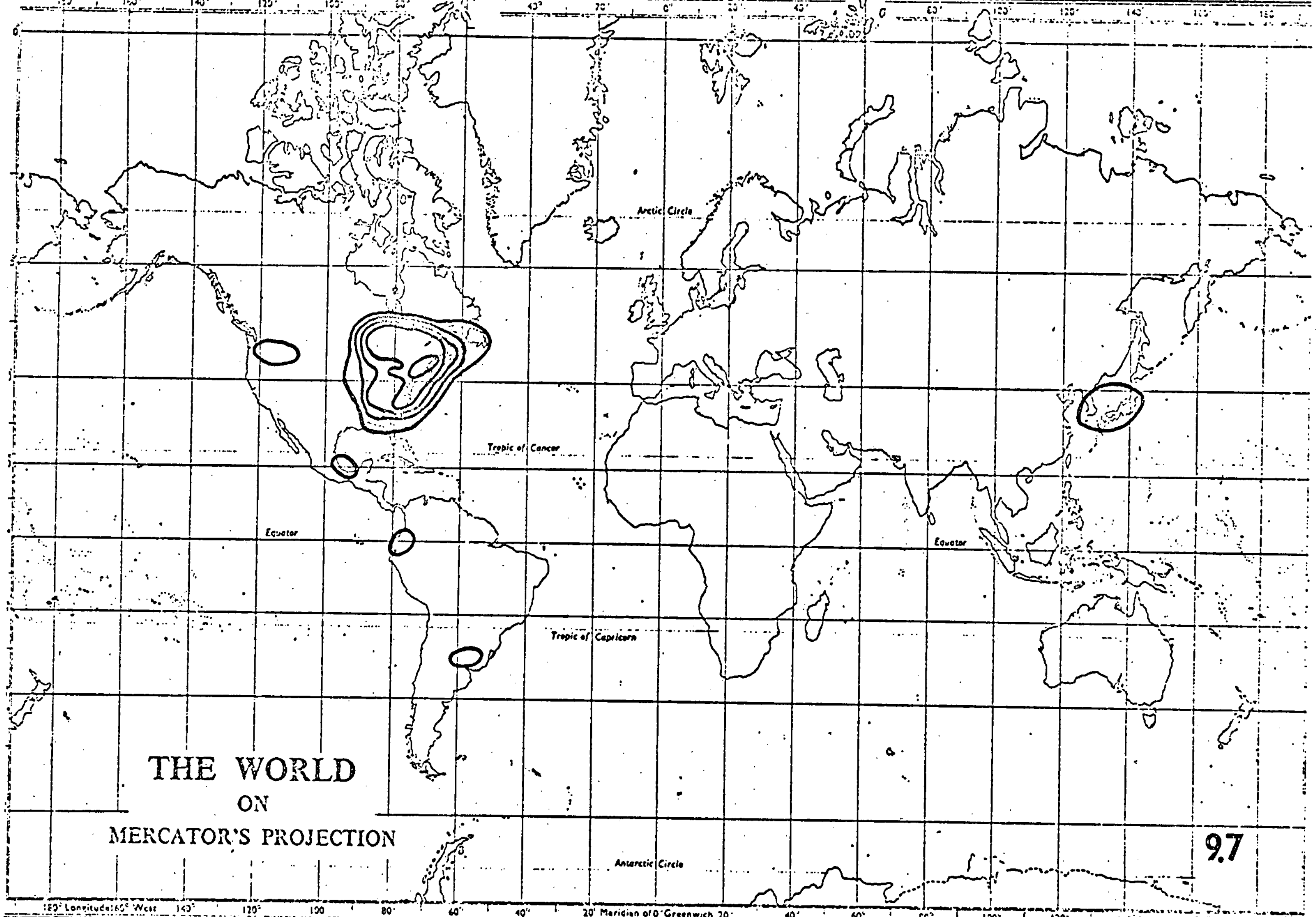
Tropic of Cancer
Equator
Equator
Tropic of Capricorn
Antarctic Circle
Meridian of 0 Greenwich
Longitude West
Longitude East


THE WORLD
ON
MERCATOR'S PROJECTION
Arctic Circle
Tropic of Cancer
Equator
Equator
Tropic of Capricorn
Antarctic Circle
9.6



Arctic Circle
Tropic of Cancer
Equator
Equator
Tropic of Capricorn
Antarctic Circle
THE WORLD
ON
MERCATOR'S PROJECTION
9.7

The South American species have a more northerly distribution.

It is obvious that the sections of the Vesicariae complex as outlined by Kükenthal have a series of quite distinctive distribution patterns. In particular, Sects. Vesicariae, Lupulinae and Tentaculatae have very different distributions from the rest, and the Vesicariae themselves have a very distinctive distribution. Interpretation of the significance of these distributions is, however, very difficult in the absence of information on even the chromosome numbers of most of the Southern Hemisphere and Eastern Asiatic species, but they would certainly seem to imply distinctive evolutionary pathways in the different sections. The Eastern Asiatic/Eastern North American distributions of the Tentaculatae and Lupulinae appear to be very similar to the distributions of a large number of species and genera in the North American and Asian floras. The major difference from these is the presence of South American representatives in both sections. Only one species (C.michauxiana) in the Lupulinae is common to both areas. This type of distribution has been widely interpreted as being a relict of a much more extensive distribution area which included the Bering Straits area and other parts of North America and Asia (cf. Li, 1951). A number of genera are known to have had much more northerly areas and fossil evidence has been found to support this interpretation, e.g. in Liriodendron (cf. Good, 1964, Fig.74). It therefore seems likely that the two sections with this distribution were probably once much more widespread and are probably comparatively old. The disjunct distributions of the Pseudocypereae and the Paludosae suggest that these are probably also old groups which may once have had more extensive, continuous distribution types, but it is extremely difficult to separate the effects of long distance dispersal and relict disjunctions.

Koyama (1959) has suggested that C.trifida and C.pumila in the Paludosae reached South America by long distance dispersal on ocean currents. Both of these species have buoyant propagules and are usually halophytic, but this does not apply to all the South American species of this section or to the members of other sections represented there. In the majority of cases, it seems more likely that migration has taken place from North to South America, along the mountain chain of the Andes. This is especially true in the Lupulinae and Tentaculatae which have no Australasian species (although the possibility that some did occur and are now extinct cannot be entirely ruled out). In any case, the presence in Australasia and South Africa of species belonging to the Paludosae and Pseudocypereae still has to be explained. It seems most unlikely that these distributions could be the result of long range dispersal alone, and it therefore seems necessary to assume that these sections were at some period more widespread and had more continuous distributions. The Vesicariae, in contrast, have a continuous distribution which shows no strong disjunctions in any part of the range. The species responsible for the widespread parts of the section's range are themselves almost completely circumpolar. This type of distribution would suggest that the group is comparatively recent and may not yet have spread to its full potential distribution area. Interpretation of the distribution is, however, extremely difficult without some other indicator of phylogeny.

The use of distributional data alone can rarely indicate evolutionary pathways and centres of origin since it is almost always possible to suggest several alternative migration routes and evolutionary centres on the basis of a single set of distributional data. Abundant examples are available in the literature where authors using the same distribution data have come to exactly opposite conclusions when trying to determine centres of origin and other geographical and evolutionary pathways. While a plausible hypothetical evolutionary and geographical history might be constructed for the Vesicariae complex, it would be almost impossible to confirm this without data from the Southern Hemisphere and Asiatic species: at present this is almost completely lacking. The only conclusions which seem valid in the light of current information are that the sections have widely differing geographical distributions which presumably reflect different evolutionary pathways, and that they can be divided into three groups on the basis of their geography as follows:

1) Sects. Lupulinae and Tentaculatae - with disjunct E. North American/E. Asian distributions and a few representatives in S.America.

2) Sect. Vesicariae - with a circumboreal/north temperate and no Southern representatives.

3) Sects. Pseudocypereae, Paludosae and Hirtae - with much wider, disjunct distributions and numerous Southern Hemisphere representatives.

The Hirtae could possibly be considered as a separate group since they have a more continuous Eurasian/North American distribution and have southern species only in South America.

9.2.6 Conclusions

The evidence from the present study and from published material strongly suggests that there is considerable justification for maintaining the present practice of dividing the species contained in the Vesicariae complex into a series of separate sections. The main reasons for doing so may be summarised as follows:

1) Sect. Pseudocypereae differs from the Vesicariae in having lower chromosome numbers and C.pseudocyperus does not form viable hybrids with C.vesicaria, suggesting a fundamental discontinuity between the two sections. C.pseudocyperus is also anatomically distinct from members of the Vesicariae and the two sections have very different geographical distributions. The section also appears to differ from the Hirtae for similar reasons although their geographical distributions are not so distinct.

2) Sect. Hirtae differs from all the other sections in the complex in having a very wide range of chromosome numbers. Crosses with the Vesicariae produced no fertile seeds. The section is anatomically diverse, but none of the species closely resembles members of the Vesicariae (although C.lasiocarpa is probably closer than the rest). In view of the anatomical and cytological diversity of this group, it is possible that it is heterogeneous, but this needs to be investigated in greater detail. The distribution of this section is quite different from the Vesicariae.

3) Sect.Paludosae has a chromosome number range very similar to the Vesicariae. Anatomically, however, it is distinct and its geographical distribution also separates it from the latter section. No biosystematic evidence is available at present.

4) Sects. Lupulinae and Tentaculatae were not investigated in detail, but their chromosome numbers seem to indicate that they are distinct, particularly with regard to the former section which has the lowest chromosome numbers in the whole complex. Anatomically, however, the Lupulinae show strong similarities to the Vesicariae, suggesting a closer relationship, but the geographical distributions of both of these sections are very restricted, although both reach South America.

It would appear that the present evidence favours maintenance of the Vesicaria, Hirtae, Pseudocypereae and Paludosae as separate sections although they form a closely related group within subgenus Carex. The position of Sects. Lupulinae and Tentaculatae is uncertain since these have not been investigated in detail, but morphologically and anatomically the Lupulinae at least are closer to the Vesicariae than any of the other sections in the complex.

At present, the data available from chemotaxonomic studies are not sufficiently complete to allow any firm conclusions, but the results contradict those obtained from other methods in suggesting that C.pseudocyperus, at least, is very similar in its protein content to C.vesicaria and C.saxatilis, while C.rostrata shows a rather different banding pattern. A more extensive chemotaxonomic survey is required before these problems can be resolved.

It must be admitted that much of the evidence presented in the above account is very incomplete and that much more extensive surveys are needed to provide a fully convincing account of the sectional delimitation in the Vesicariae complex. Since the present study was concerned primarily with British, and to a lesser extent N.W. European species, it is inevitable that many of the conclusions must be tentative: a large proportion of the species of all the sections considered are found outside Europe. In any future study of sectional delimitation in this group, it would be necessary to obtain samples of species from a number of different regions to ensure that the survey is more comprehensive and to demonstrate that any apparent discontinuity between sections implicates a high proportion of their constituent species. It is also likely that a more complete survey will raise more problems as many of the sections in Carex were based originally on European and W.Asian material and have not been critically revised in the light of material from other regions. There is still considerable doubt surrounding the exact affinities of a number of species, e.g. Reznicek & Ball (1974) consider that C.retrorsa should be transferred from the Vesicariae (they include the Tentaculatae in the Vesicariae) to the Lupulinae. These uncertainties must also be borne in mind when the significance of chromosome number ranges and geographical distributions is being considered.

In general, however, the use of a mixture of experimental techniques appears to present a valuable method of tackling problems concerned with intrageneric classification in Carex. Although the use of a single technique such as chemotaxonomy may allow a much wider survey to be carried out, it is often difficult to assess the reliability of the results obtained without supporting data from other sources. If the techniques employed in the present study can be applied to a wider range of species and sections within Carex and the Cyperaceae, it may be possible to obtain a much clearer understanding of the evolutionary development of these difficult groups.

9.3 Variation patterns in the Vesicariae.

The second problem under investigation was the nature and the sources of the widespread variation noted in most of the members of Sect.Vesicariae. This has been investigated mainly in C.rostrata. where the observed variation patterns appear to be the result of a complex of environmental and genetic factors. These are discussed under two main headings - sources of local variation and large scale geographical variation.

9.3.1 Sources of local variation (cf. Chapter 8 - Sections 8.3.1 and 8.3.2.)

The two major sources of variation on a local scale appear to be environmental factors and intraclonal genetic variation. The former contributes to the considerable range in size which can be seen in most stands of C.rostrata. Water depth in particular has a marked effect, although it is not known whether this may be brought about by a combination of physical and chemical factors such as oxygen concentration in addition to any direct effect which water depth might have by itself. Nutrient levels also affect plant size markedly, but as far as can be ascertained from the evidence available, most of the shape characters measured, including utricle and glume shape in addition to leaf shape, appear to be comparatively unaffected even though the general size of the plants may vary considerably. The major factor contributing to shape variations appears to be the genetical differences which occur between the different vegetative clones within a population. Evidence from the large C.rostrata population at Threipmuir suggests that many clones may be quite distinctive in their morphological characteristics, and although the clonal nature of these variations has not yet been confirmed by biosystematic studies, there would appear to be little doubt that a considerable part of the variation noted was genetically determined as different morphological types grow side by side in an apparently homogeneous environment. C.rostrata is apparently an outbreeding species and it seems likely that a large part of the small scale variation can be interpreted as the result of extensive cross fertilisation, recombination and selection over a very wide area. The morphological differences between individual clones are emphasized by the comparative uniformity of the clones themselves, and by the ability of individual clones to grow to large sizes. The high variability of C.rostrata on a local scale therefore appears to be the result of a combination of size differences caused by environmental factors and shape differences caused primarily by genetic variation between clones. No obvious examples of clinal variation have been found and there is no evidence for apomixis.

The situation in C.vesicaria and C.saxatilis may be similar but it seems likely that the species will differ in the amount of outcrossing which occurs, and in other details of their reproductive biology. These species generally seem to be more variable on a large scale, with numerous geographical races and variants, and are less variable at a local level. This, however, needs to be confirmed by more detailed investigations.

Another possible variation source which has been considered is hybridisation and introgression. At present, the evidence for widespread introgression is very limited. The studies carried out on an apparent hybrid swarm at Grantown-on-Spey (Section 8.4) suggested that three main groups were present in the sample collected:

1) typical C.vesicaria plants.

2) plants which were almost exactly intermediate between C.rostrata and C.vesicaria in morphology and epidermal anatomy. These were almost certainly F_1 hybrids and were sterile or semi-sterile.

3) a few plants which were very close to typical C.vesicaria in their morphology but possessed a few C.rostrata characters in their epidermal structure. These were fertile and assumed to be backcrosses.

The occurrence of backcrosses suggests that gene flow is possible in one direction at least, but no material of C.rostrata was collected at this site, and it is not possible to determine whether gene flow can also occur in this direction. Morphological evidence therefore suggests that introgression is possible, but it is very difficult to estimate the importance of this process as a variation source. The evidence available indicates that its effects are probably very local and therefore seems to rule out introgression as an important source of variation. Two points must be borne in mind, however. If hybridisation occurs as widely as some reports indicate, (e.g. Kreczetowicz, 1935), then even a low rate of introgression might lead to substantial gene flow over the total area of overlap of the two species. Another difficulty is raised by the possibility that some form of differential introgression might occur . Levin (1975) found that in two species of Phlox, the flow of alleles from one species into another was not even , and that some alleles were selected for while others were not. It is therefore possible that genes controlling obvious morphological characters might not be transferred by introgression while less obvious physiological genes might be exchanged between populations quite rapidly. In this case, it would be impossible to detect introgression by morphological techniques and even studies of isoenzyme systems would not necessarily show that this process is occurring. As far as morphological variation is concerned, however, introgression is unlikely to be a major variation source in C.vesicaria at least. Hybridisation between C.saxatilis and C.vesicaria is dealt with in section 9.4. Other hybrid combinations are possible but they are unlikely to be significant as a variation source since they normally appear to be much rarer than C.vesicaria x rostrata.

9.3.2 Regional variation in C.rostrata.

While the small-scale local variation described above forms a more or less continuous variation range between morphological extremes, the larger scale variations are discontinuous and require some form of taxonomic recognition. The taxonomic history of the large, wide leaved North American forms of C.rostrata has been rather complex. Boott (1839) considered that they were sufficiently distinct to warrant specific rank. The characters which he used to separate them were mainly the width of the leaves which are usually septate nodulose, the longer female spikes and utricles, and the more acute or even awned female glumes. The taxon has subsequently been reduced to varietal rank and has been treated as such by Fernald (1942, 1950). The most commonly accepted name at this rank has been C.rostrata var.utriculata (Boott) Bailey, but Fernald (1942) points out that the nomenclaturally correct epithet at this rank would appear to be var.minor, since Boott had also described C.utriculata var.minor (Boott, 1839). MacKenzie (1931-35) did not recognise any subspecific taxa and included all the North American material in C.rostrata. This treatment has also been followed by Hermann (1970).

To a European botanist, it seems highly unsatisfactory to include all of the North American material in C.rostrata without some formal taxonomic recognition being given to the differences between the forms occurring in each continent. Extreme forms of the North American plant are very distinct and would certainly seem to warrant full specific rank. The difficulties are caused by the occurrence of numerous intermediates which have never been successfully assigned to two taxa. The commonly accepted practice of recognising var.utriculata, although it acknowledges the presence of intermediates, also seems unsatisfactory since there are a number of distinctions between the two forms which suggest that a higher rank would be more appropriate for the North American plants. The main reasons for this suggestion are:-

1) Morphology (cf. Section 8.3.3). Although it is almost impossible to find simple characters which separate the two forms completely, it seems that it is possible to distinguish the bulk of

the material by use of composite characters produced by a principal components analysis (PCA). It seems likely that use of a discriminant function would allow the majority of specimens to be assigned to one taxon or the other.

2) Anatomy (cf. Sections 5.4.1 & 8.3.3.) Almost all of the American var. utriculata material had a smooth adaxial epidermis and abaxial stomata. North American plants with anatomical characters resembling the European forms were smaller than the 'utriculata' types and were generally intermediate or closer to the European forms in their morphology. They are found only in the Northern parts of the continent and do not extend as far South as the utriculata forms. Most of the large specimens from Siberia were identical to the North American material in their anatomy. In addition to the differences in epidermal structure, the utriculata forms usually had a well-developed hypodermal layer below the bulliform cells of the leaf. This character was very rare in European material, only being found in a few of the broad-leaved forms but these were otherwise similar to the type variety. The North American forms also differ in culm anatomy, having a more irregularly shaped culm section and various other small differences, although in this case the large European forms match the American variety more closely.

3) Cytology. The only two chromosome counts available for var. utriculata both gave 2n = 82 (Wahl, 1940). Nearly all the counts for European material have given 2n = 76, and no counts are reported for var. rostrata from North America (cf. Sections 6.3.1 & 6.5).

As the differences between the forms are generally correlated with geographical differences, it would seem necessary to give the North American forms the rank of sub-species or species. I do not intend to propose any formal change of rank as a more detailed study of the morphological, anatomical and cytological differences between the forms is required, along with a thorough biosystematic and chemotaxonomic study before any final conclusions can be reached. In particular, the status of the intermediate North American plants needs to be determined. Unless it is possible to find a consistent set of morphological characters which can be used to separate var. utriculata and C.rostrata with reasonable certainty, it is doubtful if species rank could be succesfully applied to this taxon, in spite of any genetic discontinuity which might be implied by the difference in their chromosome numbers. As Merxmüller (1963) points out, it is only practicable to use morphological characters for routine herbarium identification, and without a good correlation with morphology, species based on biosystematic criteria or on very cryptic characters are very unlikely to be widely accepted because of the difficulties encountered in trying to make a correct identification. At present it is not clear to what extent the morphological differences found in this study can be used to discriminate between the two forms, although the results of the PCA are certainly promising. It would appear that the two taxa are morphologically very similar and have more or less parallel variation ranges in size and shape, producing a very confusing complex of phenotypes which are easily misclassified. The epidermal types apparently offer a clear-cut distinction between the two taxa and these can be checked easily and quickly by use of cellulose acetate impressions, but the correlation between epidermal anatomy and morphology needs to be investigated in more detail, over a wider sample, to determine the reliability of this character. The use of subspecific rank would alleviate these problems to some extent since some degree of overlap between subspecies is expected and the presence of numerous intermediates would therefore be slightly more acceptable. As a bonus, this would remove the nomenclatural problem surrounding the use of varietal rank, as the epithet var. minor need not be used at a new rank and no subspecies have been proposed to distinguish the North American forms. The cytological and geographical evidence, however, suggest that restoration of var. utriculata to full specific rank would probably be the most satisfactory solution in biosystematic terms. The anatomical differences between the two forms seems to be at least as great as those used by Kukkonen (in Robson et al., 1970) to separate C. microglochin and C. camptoglochin (though Moore & Chater (1971) consider that these are only worthy of subspecific rank). Without further data and studies of living material, it is impossible to give a satisfactory taxonomic decision on this point and I have therefore avoided making any formal nomenclatural changes at the present time.

The total distribution range of C. rostrata var. utriculata is not known, as there is considerable confusion caused by the occurrence of large, broad-leaved forms of C. rostrata s.s. The latter have epidermal structures identical with the normal small forms of the type variety and therefore seem to be quite distinct from var. utriculata which is probably restricted to North America and Eastern Asia. I have not seen any specimens of var. utriculata from Greenland, although Fernald (1942) considered it to be present there. Kreczetowicz (1935) gives a range which includes European Russia and Scandinavia, but it seems likely that his concept of this taxon, like Kükenthal's, was rather too wide and included the broad-leaved European forms of C. rostrata s.s.

As noted in Chapter 8, both C. vesicaria and C. saxatilis apparently possess at least two chromosome races which could possibly be correlated with the numerous morphological variants described for these species. A thorough investigation of the taxonomy and biosystematics of these species would be of considerable interest and would probably help to clarify evolutionary relationships within the Vesicariae.

9.4 The origins of C.grahamii

A number of suggestions have been made concerning the origins and status of C.grahamii and taxonomic treatments have varied accordingly, and are summarised below:-

1) a separate species, probably closely related to C.saxatilis (Boott, 1845; Dandy, 1958).

2) a hybrid between C.saxatilis and C.vesicaria (Jermy & Tutin, 1968; Dandy, 1969).

3) a hybrid between C.saxatilis and C.hostiana or C.binervis (Clapham, Tutin & Warburg, 1962)

4) a variety of C.vesicaria (Kükenthal, 1909)

5) a variety of C.saxatilis (Hooker & Arnott, 1860)

To what extent does the evidence available support any of these suggestions?

Morphologically, the British material of C.grahamii from Meall nan Tarmachan and Ben More is almost exactly intermediate between C.saxatilis and C.vesicaria, strongly supporting the second suggestion, (cf. Section 8.5). Material from Glen Clova is closer to typical C.saxatilis material and there are numerous large forms of the latter which form an almost complete series of intermediates between the two. The Scandinavian material is similar, but more variable, and again there is a range of material from large C.saxatilis plants to forms which are almost exactly intermediate between C.saxatilis and C.vesicaria. The occurrence of three stigmas in many of the female flowers of C.grahamii would certainly seem to indicate that hybridisation with a three-stigma species has taken place, but the genetic basis of this character is not known. It is possible that a simple mutation could be responsible, producing a change which is of very little phylogenetic significance, but the same objection could be raised for the majority of characters used in plant taxonomy. The simplest interpretation of the morphological evidence would suggest that C.grahamii consists of a series of F_1 hybrids between C.saxatilis and C.vesicaria, together with a series of backcrosses between the hybrid and C.saxatilis. Both the Meall nan Tarmachan and Clova populations consist of single clones and it is likely that most of the remaining British clones are similar. There is no morphological evidence to suggest that any other species (e.g. C.rostrata, C.binervis and C.hostiana) might be one of the parents of the hybrid.

Anatomically, C.grahamii is again intermediate between C.saxatilis and C.vesicaria and the Tarmachan and Clova populations are similar, although a few minor differences occur (cf. Section 5.4.4). The close morphological resemblance between the Clova clone and the former species is supported by the larger adaxial epidermal cells of this clone. The Tarmachan material shows a smaller difference in size between adaxial and abaxial cells and is thus more similar to C.vesicaria. There is no anatomical evidence to support the involvement of C.rostrata, C.binervis or C.hostiana in the ancestry of C.grahamii.

Cytologically, C.grahamii shows a number of peculiarities, (cf. Section 6.6). The diploid number is 2n = 82 or 84 and either two large bivalents or two trivalents are present in the complement. The observed chromosome number would seem to rule out any possibility that C.rostrata, C.binervis or C.hostiana could be potential parents in a cross with C.saxatilis, as they would be expected to produce diploid numbers of 2n = 78, 77 and 68 respectively. It is possible that an increase in chromosome number could be brought about by chromosome splitting, but this seems unlikely as a minimum of four bivalents would have to be involved, even in the simplest case (2n = 78 to 2n = 82). The more or less regular chromosome pairing which occurs in C.grahamii together with the chromosome number which is higher than would be expected from a C.saxatilis x vesicaria cross (2n = 81 = 40 + 41), suggests that this taxon might be better treated as a separate species, but there are some objections to this proposal. Although it is possible that the two large bodies seen on metaphase plates were bivalents, it is more likely that they were trivalaents since they are similar in size and shape to the chain trivalents found by Faulkner (1972) in members of Sect.Acutae. In addition, the chromosome number of 2n = 83 obtained for a C.grahamii x vesicaria artificial hybrid (cf. section 6.3.3) would suggest that the gametes were n = 41 and n = 42, in agreement with a number of 2n = 84 for C.grahamii (C.vesicaria has 2n = 82). It is therefore highly likely that there are two trivalents in the metaphase I stage: these would not be expected in a separate species with a normal breeding system. An additional objection is that chromosome pairing in the artificial hybrid mentioned above was remarkably regular, with only one trivalent present, indicating that there is a strong genetic affinity between the two taxa. If C.grahamii is maintained as a separate species, then irregularities in chromosome pairing at meiosis in hybrids is minimal and confined to one or two trivalents in the complement. This would effectively remove the major reason for considering C.grahamii as a separate species in the first place.

This leaves three alternative explanations of the origin of C.grahamii; it is either a hybrid between C.saxatilis and C.vesicaria or a variety of one of these two species. The cytological evidence in this case is less helpful since some modification of the karyotype must be postulated, whichever of the three possible explanations is accepted. It seems most likely that a hybrid origin is responsible for the limited cytological abnormality shown by C.grahamii, as it is difficult to explain the presence of trivalents or large bivalents in a variety of either of the other two species. It would be possible to explain the presence of two trivalents in a C.vesicaria complement by assuming that trisomy has occurred for two chromosomes, but a more extensive karyotype modification would be needed to produce the C.grahamii complement from C.saxatilis. Since, however, morphological evidence seems to suggest a stronger connection between the latter, it seems preferable to regard the karyotype of C.grahamii as a modification of a hybrid between the other two species. Crossing behaviour seems to indicate that C.grahamii is equally interfertile, and forms F_1 hybrids, with both species. F_1 hybrids between C.saxatilis and C.vesicaria have been obtained (Chapter 7 - Section 7.3.2) but these have not flowered and it has therefore been impossible to investigate their floral morphology and meiotic behaviour. The vegetative morphology of these plants is, however, very similar to that observed in C.grahamii. The apparent sterility of the C.grahamii clones has been interpreted as a strong indication of a hybrid origin, but this may not be as reliable as has been assumed. Plants from the Tarmachan clone readily set seed when pollinated with pollen from other British members of the section, and there is no obvious impairment of fertility when pollen production is examined. Counts of fully swollen, stainable pollen grains in C.grahamii were always 85% or higher. As a self-incompatibility system is present in both C.vesicaria and C.rostrata it is possible that self-incompatibility of isolated clones may be responsible for the sterility of C.grahamii, rather than meiotic and fertility disturbance in a hybrid. Inflorescences of C.grahamii left uncovered and allowed to self pollinate in the greenhouse set no seeds although pollination certainly did occur.

On the basis of morphological and anatomical evidence, it seems best to regard C.grahamii Boott as a mixture of F_1 hybrids between C.saxatilis and C.vesicaria and a series of backcrosses with C.saxatilis. The cytological evidence is more equivocal, and does not provide any conclusive reason for accepting this explanation as it is necessary to postulate some changes in karyotype immediately before or after the hybridisation to account for the observed chromosome number and behaviour. Chromosomal rearrangements are also required, however, for all of the other possible explanations and a hybrid origin of C.grahamii seems to be the most satisfactory interpretation of the data available. The evidence from crossing experiments is quite compatible with a hybrid origin, although it is at present far from complete. The correct name for this taxon should therefore be C. x grahamii Boott (= C.saxatilis x vesicaria). There is no evidence at present to suggest that any other species is implicated in the origin of the hybrid.

One difficulty which arises is that a C.saxatilis x vesicaria cross is somewhat unlikely since, in Britain at least, the two species are normally strongly separated by their ecological preferences and rarely grow close together under present day conditions. The most likely explanation is that both species may have been widespread and less strongly separated in the glacial and post-glacial periods.

C.vesicaria is recorded for the interglacial period, but no records for C.saxatilis are available (Godwin, 1956). The records given by Godwin indicate that C.rostrata was present from the interglacial period and through the whole of the glacial and later periods. If habitats **were closer during the ecological upheaval** of the post-glacial period, it is possible that widespread hybridisation could have occurred and that C. x grahamii consists largely of postglacial relicts. There is at present no fossil evidence such as intermingled fruits of the two species to support this conjecture. In Scandinavia, C. x grahamii appears to be more widespread and the two species occupy a considerable area of overlap (Hulten, 1950) but again the ecological separation of the species appears to be quite strong. In some of the sites such as the Loch Tay area, although there is a considerable vertical separation of habitats, the horizontal distances between present day populations of the two species are not very large (ca. 2 km.). It is possible that occasional cross-pollination could occur by means of wind blown pollen, but successful production of hybrid seeds followed by establishment of hybrid plants must be very rare. Backcrossing with C.saxatilis could, however, be relatively easy. The Ben More and other western populations of C.grahamii are probably also reasonably close to both parental species, but the Glen Clova clone is very isolated and only very small amounts of C.saxatilis occur in the same area. C.vesicaria appears to be completely absent from the immediate vicinity.

It must be emphasised that much of the foregoing discussion has been based on work carried out on the Meall nan Tarmachan clone, and more detailed studies of the Glen Clova and other clones are required before the problem of the origin of C.grahamii can be regarded as fully resolved. Difficulties in obtaining flowering material have made it impossible to obtain chromosome counts for the Glen Clova and Ben More clones and no crossing experiments have been carried out for the same reasons. It would, however, be extremely interesting to have data for the other populations and if this proves to be possible, it should remove any remaining doubts surrounding this taxon. A study of flavonoid components might also help to resolve some of the ambiguity in the cytological and biosystematic evidence.

9.5 A final comment on experimental techniques.

There can be little doubt that the use of a range of experimental techniques is an extremely effective approach to taxonomic problems. An increasing number of publications now report the routine use of a wide variety of techniques in addition to traditional taxonomic methods and it is obvious that these are becoming ever more essential in our attempts to understand the processes which occur in the evolution of living organisms. The results obtained in the present survey certainly suggest that the use of experimental techniques in *Carex* offers a vastly increased data base which can be used to interpret taxonomic situations which cannot be investigated adequately by traditional methods.

As might be expected the increasing use of experimental techniques is not entirely free from problems. One major difficulty is the length of time required for many of the investigations. Biosystematic studies are especially time-consuming as production of flowering F_1 plants and later generations may require a period of several years in perennial species. Because the number of crosses carried out to obtain a complete crossing table increases enormously as the number of species to be tested increases, there is a limit to the number of plants which can be studied by these techniques in any reasonable period of time. As a result of this limitation, it is impossible to sample a wide range of material from each species and biosystematic studies are, with few exceptions, generally very incomplete in comparison with the intensity of sampling which is possible in most morphological studies. It may also be difficult to obtain supplies of living material of species which grow in remote or inaccessible areas. Similarly, collection of sufficient quantities of seeds or other organs for chemotaxonomic studies may pose problems, and it is often impossible to ensure that a widespread species is adequately sampled over its whole range. In the present study, time limitations have been a major difficulty: it has not been possible to follow up the results of the biosystematic experiments in the time available and many of the other surveys are incomplete. This is, however, inevitable in a research project of fairly limited duration. The conclusion to be drawn would seem to be that the taxonomist wishing to carry out a thorough experimental taxonomic survey must resign himself (or herself) to a long-term project which will occupy several years. A better approach might be to encourage the formation of interdisciplinary teams which are capable of investigating several different aspects of a problem simultaneously and thus reduce the time required.

In spite of these reservations, however, experimental techniques can produce a considerable body of information in a comparatively short time and they are certain to become even more important in future investigations. It seems most unlikely that the evolutionary development of difficult groups such as the Cyperaceae can ever be successfully interpreted without the aid of modern experimental techniques, although there is still considerable scope for investigations of morphological characters in many groups, especially if improved instrumentation such as the scanning electron microscope can be applied. Although the more extreme claims of some of the numerical taxonomists have raised indignant protests in many parts of the taxonomic community, the value of many of the techniques which they have devised is becoming more widely recognised, and these should enable taxonomists to evaluate much of the data being produced by the more recent techniques in a more meaningful way. It is to be hoped that the extensive improvements in taxonomic methods which have appeared in the last few decades will lead to a corresponding increase in our understanding of the organisms which we study.

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APPENDIX 1 : SOURCES OF MATERIAL

1) Collecting sites

V.C.28 West Norfolk, nr. King's Lynn. C.hirta Pl.

V.C.73 Kirkcudbrightshire, Loch Ken. C.vesicaria Pl. S.+

-do- Cree Wood, nr. Newton Stewart. C.vesicaria Pl.

V.C.83 Midlothian, Threipmuir Reservoir, nr. Balerno. C.rostrata P. Pl. S.

V.C.88 Mid Perthshire, Loch Tay, nr. Killin. C.vesicaria P. Pl.;
C.rostrata Pl.

-do- Meall nan Tarmachan. C.grahamii P. Pl.;
C.saxatilis P. Pl.

V.C.90 Angus, Glen Clova. C.grahamii P. Pl.

V.C.95 Moray, Knockando, nr. Nether Tamdhu fm. C.rostrata P. S.

-do- -do- C.aquatilis, C.binervis, C.flacca, C.panicea,
C.laevigata, C.pilulifera, Eriophorum,
angustifolium S.

Norway: Sör Tröndelag: Oppdal, Kongsvoll. C.saxatilis S.

Switzerland: Valais, nr.Zermatt, C.muricata S.

Canada: Labrador, Fraser Canyon, nr. Nain. C.saxatilis P.

P. - population sample

Pl. - live plants

S. - seeds

+ - collected from plants grown in a greenhouse.

2) Botanic gardens and other sources

Seeds of C.pseudocyperus were obtained from the following Botanic gardens:

Liege, Belgium

Aarhus, Denmark

Halle, Germany.

Plants of C.pseudocyperus were obtained from Mr. M. Feesey.

Seeds of C.grayii were obtained from the following botanic gardens:

Glasgow, U.K.

Bangor, U.K.

Nancy, France.

Tübingen, W.Germany.

3) Herbarium specimens used in PCA

a) C.rostrata var. utriculata.

U.S.A. : Alaska, Valdez 1 viii 1935 F.P. Anderson 2886 (K).

-do- : -do- nr.Anchorage 20 vi 1966 M. Williams 1559 (K).

Canada : British Columbia, Vancouver I., Longford Lake 29 vi 1887 J. Macoun (K).

-do- : -do- Norfolk Sound 2 viii 1938 T.R.G. Moir 130 (K).

-do- : North West Territories, MacKenzie Dist., Fort Simpson I. 1 viii 1955 W.J. Cody & J.M. Matte 9115 (K).

-do- : Alberta, Wood Buffalo Park, Moose Lake 8 viii 1929 H.M. Raup 1833 (K).

-do- : Saskatchewan, ? Bourgeau 26 vii 1858 F. Boott (K).

-do- : Manitoba, Norway House, N. end of Lake Winnipeg 24 vi 1948 H.J. Scoggan 2854 (K).

-do- : Ontario, Kapuskasing, Cochrane Dist., Girvey 26 vi 1953 W.K.W. Baldwin 4897 (K).

-do- : Quebec, Ile d'Anticosti, Crique de la Chaloupe 16 viii 1926 F.T. Marie-Victorin & R. Germain 25198 (K).

-do- : -do- Marin Heights, Argenteuil 18 vi 1942 F.T. Marie-Victorin & R. Germain 55258 (E).

Canada : Labrador, Goose Bay, Terrington Basin 29 vii 1950 J.M.Gillet & W.I. Findlay 5502 (K).

-do- : Nova Scotia, Halifax Co., Musquodoboit 3 viii 1930 J.Rousseau 35300 (K).

-do- : -do- Guysborough Co., Conso 14-16 viii 1930 J.Rousseau 35504 (K).

++ U.S.A. : Idaho, Ouijhee Co., Rock Creek at Triangle 12 viii 1951 W.F.Baker 8575 (2) (K).

-do- : California, Sierra Nevada, S.Fork of S.Joaquin River vii 1900 H.M. Hall & H.P. Chandler 625 (K).

+ -do- : -do- Nevada Co., Truckee 7 viii 1903 A.A. Heller (K)

-do- : Nevada, Washoe Co., Hunter Creek 2 viii 1912 P.P. Kennedy 1899 (E)

+ -do- : Colorado, Gunnison 23 vii 1901 C.F. Baker (K).

-do- : -do- W.Pagosa Peak viii 1899 C.F. Baker (K).

+ leaf and/or culm anatomy examined.

++ specimen K38 (referred to in text - Chapter 5).

b) C.rostrata (papillate N.American forms).

Canada : North West Territories, Bear Lake, Cumberland House Fort, ? Richardson (K).

-do- : Quebec, Gaspé Co., Table-Top Mt. 4 viii 1906 M.L. Fernald & J.F. Collins 189 (K).

-do- : -do- Montane Co., Mt. Logan 9 viii 1948 P. Levesque 48432 (K).

-do- : Newfoundland, Wild Cove, Humber Dist. 5 vii 1950 E.Rouleau 581 (E).

-do- : -do- W.of St.Anthony 8 viii 1951 D.B.O.Saville 2780 (K).

-do- : Nova Scotia, St.Paul I., Lena Lake 23 vii 1929 L.M.Perry & M.V. Roscoe 126 (K).

Greenland : Majut, 61° 04'N 45° 35'W 2 ix 1962 Hansen, Hansen & Petersen (E).

c) C.grahamii

Scotland : Mid Perth, Ben More vii 1905 Wilson & Wheldon (7) (BM)

-do- : -do- -do- 26 vii 1889 E.S. Marshall 942 (2) (BM).

-do- : -do- -do- 16 vii 1910 E.S. Marshall 3481 (2) (BM).

-do- : -do- -do- 16 vii 1910 E.S. Marshall 3482 (3) (BM).

-do- : -do- -do- 21 vii 1938 E. Nelmes 410 (4) (K).

-do- : -do- -do- 8 ix 1900 J. Fraser (3) (K).

-do- : -do- Meall Ghaordie 27 vii 1891 E.S. Marshall (7) (BM)

-do- : -do- -do- 27 vii 1891 E.F. Linton (2) (BM)

-do- : -do- -do- towards Creag Loaghan . ?

E.F. Linton (BM)

-do- : -do- Glen Lyon, side of Meall nan Tarmachan 8 vii

1925 D.M. Haggart (BM).

-do- : Angus, Glen Clova, banks of White Water vii 1832 J.MacNab (E)

Norway : Sör Tröndelag Oppdal, Kongsvoll vii 1885 P. Olssen (BM)

-do- -do- -do- -do- 23 vii 1886 Herb.

H. Gravnik (3) (BM)

Sweden : Lapponia Lulensi 1864 Herb. H. Hance 12735 (BM).

-do- : -do- -do- ? N.J. Anderson (BM)

-do- : -do- Tornensis; ? Riksgravsen 20 vii 1907 E.Warodell (BM)

-do- : Torne Lappmark : Jukkasjärvi, Kappastjarno 7 viii 1954

C.G. Alm 2198 (BM).

-do- : -do- -do- Jukkasjärvi, Vassijaure 12 viii 1956

C.G. Alm 2837 (BM).

-do- : -do- -do- Jukkasjärvi, Björkstugen 18 viii 1956

C.G. Alm 2896a (BM).

-do- : Åsele Lappmark : Åsele viii 1911 T.Wolfe (BM)

-do- : Jamtland : Storlieu viii 1908 J. Thorne (BM).

Sweden : Jamtland : Storlieu 16 viii 1908 E. Warodell (BM).

-do- -do- -do- 18 vii 1914 E. Warodell (BM).

-do- -do- : Are, Enafors, Högasen 20 vii 1958 H.Smith 3370 (BM)

-do- -do- -do- -do- -do- ? H.Smith 3386 (BM)

-do- -do- -do- -do- -do- 26 vii 1958 H.Smith 3424 (BM)

-do- -do- -do- -do- -do- 26 vii 1958 H.Smith 3426 (BM)

-do- -do- -do- -do- -do- 12 vii 1959 H.Smith 3546 (BM)

-do- -do- -do- -do- -do- 23 vii 1962 H.Smith 4121 (BM)

-do- -do- -do- -do- Snasahögarna 1 viii 1959 H.Smith 3736 (BM)

-do- -do- -do- -do- -do- 31 vii 1960 H.Smith 3806 (BM)

-do- -do- Handölsfjellen 9 viii 1881 E. Warodell (BM).

Finland : Enontek : ? Kilpisjaur 3 viii 1910 I. Mantell (2) (BM).

d) C.saxatilis

Scotland : Mid Perth, Meall nan Tarmachan 17 viii 1893 A.Somerville (E).

Canada : Manitoba, Churchill, Hudson Bay 30 viii 1910 J.M. Macoun Geol. Surv. Canada 79039 (E).

-do- -do- Ranken Inlet, Hudson Bay, 30 viii 1910 J.M. Macoun Geol.Surv. Canada 79040 (E).

Greenland : Ibersasak viii 1929 I.W. Hutchinson (E).

-do- : Syd Kap 71° 17'N 25° 05'W, S.Scoresby Land 31 viii 1962 D.F.C. Chamberlain (E)

-do- : Narssaq 60° 57'N 46° 02'W 30 vii 1962 K. Holmen (E)

Sweden : Torne Lappmark : Jukkäsjarvi, Tornetrask 5 viii 1953 C.G. Alm 1716 (BM).

-do- -do- Jukkäsjarvi, Tornetrask, 24 vii 1954 C.G.Alm 2052 (BM).

-do- : Lappland 24 vii 1863 Herb. Carrol (BM).

Sweden : Jamtland : Härjedalen, Starsjö, Nea Valley 11 viii 1950 H.Smith 1155 (BM).

-do- : -do- Härjedalen, Starsjö, Nea Valley 12 viii 1950 H.Smith 1170 (BM).

-do- : -do- Undersaker, Nea Valley 29 viii 1950 H.Smith 1431 (BM).

4) Examples of C.rostrata x vesicaria

v.c. 17 Surrey, Eden, Hedge Court Mill vii 1883 W.T. Beeby (BM).

v.c. 39 Staffordshire, nr. Burton-on-Trent 1861 E.Brown 1259 (BM).

v.c. 41 Glamorgan, ? Llancarach 22 v 1907 Herb. H.J. Riddelsdel (BM).

v.c. 62 North-east Yorkshire, nr. Scarborough viii 1907 J.Cryer (BM).

v.c. 64 Mid-west Yorkshire, Wharfedale, below Buckden 23 vi 1916 Herb. H.A. Maude (BM).

v.c. 69 Westmorland, nr. Coniston vii 1904 J. Cryer (BM).

v.c. 70 Cumberland, nr. Keswick 11 ix 1849 ? (BM).

v.c. 95 Moray, Grantown-on-Spey M.McCallum Webster (E)

v.c. 98 Argyll, nr. Dalmally 30 vi 1910 E.S. Marshall 3480 (BM)

In all of the above lists, the standard herbarium abbreviations are used:

Edinburgh (E)

British Museum (BM)

Kew (K)

Figures in brackets before the herbarium indicate the actual number of specimens measured.

APPENDIX 2: **FORMULAE**

1) Hoagland's Solution

10 ml. M KNO_3

10 ml. 0.25% E.D.T.A. (Fe/Na salt)

20 ml. 1/10 strength micronutrient solution+

10 ml. 0.4 M $MgSO_4$

10 ml. M $Ca(NO_3)_2$

10 ml. 0.2 M KH_2PO_4

32 ml. M NaCl

+ H_3BO_3 0.5 p.p.m.

$MnCl_2$ $4H_2O$ 0.5 p.p.m.

$ZnSO_4$ $7H_2O$ 0.5 p.p.m.

$CnSO_4$ $5H_2O$ 0.2 p.p.m.

$H_2MoO_4H_2O$ 0.5 p.p.m.

The above solution is made up to 1000 ml. with deonised water and should be diluted 10 times to give the actual strength used (1/5 normal Hoagland's solution concentration).

2) SDS Gels

a) running buffer (pH 8.3)

glycine	2.88%
Tris	0.6 %
SDS	0.1 %
β-mercaptoethanol	0.075%

b) gel buffer (pH 8.5)

SDS	0.8%)	
	)	5 ml.
Urea	24%)	

+ 5 ml. 36.3% Tris adjusted to pH 8.5 with conc. HCl added to 20 ml. ammonium persulphate (0.14%) and 10 ml. acrylamide/bisacrylamide (N,N'-Methylenebisacrylamide) (58% acrylamide, 2% bis-acrylamide). 0.025 ml. Temed (N,N,N',N',-Tetramethylethylene diamine) was added after degassing. Polymerisation takes ca.20-30 mins.

3) Urea gels

a) running buffer (pH 3.5)

acetic acid	0.9 M.
urea	6 M.

b) gel mixture

acrylamide	60%	2 parts
bisacrylamide	0.4%	
glacial acetic acid	43.2%	1 part
temed	4%	
ammonium persulphate	0.2%	5 parts
in 10 M urea		

After degassing, polymerisation should occur in 30-40 mins.

APPENDIX 3 : KEY TO THE BRITISH SPECIES OF SECT. VESICARIAE

1. Female spikes ovoid, usually less than three times as long as wide, female glumes usually less than three and a half times as long as wide. Utricles and glumes normally dark brown to black. Inflorescence usually less than 14 cm. long. Stigmas two or three. On mountains.

   2. Plant small, usually less than 30 cm. tall, utricles usually less than 3.5 mm. long (occasionally reaching 4 mm.), smooth and shiny, abruptly contracted to form a short, scarcely notched beak. Normally fertile to some extent. Female spikes up to 1.5 cm. long. .... C.saxatilis.

   2. Plant larger, up to 40 cm. or more, utricles usually more than 4 mm. long, with a ribbed, dull surface or shiny, beak distinctly bifid. If shiny then narrowly ovate and tapering gradually into the beak. (?) Always sterile. Female spikes more than 1.5 cm. long. .... C. x grahamii.

1. Female spikes cylindric, usually more than three times as long as wide, female glumes usually narrowly lanceolate or ligulate, more than three times as long as wide. Utricles and glumes light brown to straw-coloured or green. Inflorescence usually more than 14 cm. long. Stigmas three. Tall lowland plants, reaching 60 cm. or more.

   3. Utricles abruptly narrowing into beak, usually less than 6 mm. long. Leaves ± glaucous on the adaxial surface, adaxial epidermis densely papillate and bearing stomata. Female spikes usually six times as long as wide or more.
   .... C.rostrata.

3. Utricles gradually tapering into the beak, usually longer than 6 mm. Leaves normally green on both surfaces, adaxial epidermis smooth, stomata abaxial. Female spikes usually less than five times as long as wide .... C. vesicaria.

ABSTRACT OF THESIS

Name of Candidate GEORGE JOHN SHEPHERD

Address DEPT. OF BOTANY, UNIVERSITY OF EDINBURGH.

Degree Ph.D. Date AUGUST 1975

Title of Thesis EXPERIMENTAL TAXONOMY IN THE GENUS CAREX SECTION VESICARIAE.

SUMMARY

This thesis describes an experimental taxonomic study of the British species of Carex Sect. Vesicariae. A variety of techniques (anatomy, chemotaxonomy, biosystematics, cytology and morphology) were applied to three main problems:-

1. The delimitation of Sect. Vesicariae
2. The source(s) of the widespread variation noted in many of the species of Sect. Vesicariae.
3. The nature and origins of C. grahamii.

The delimitation of Sect. Vesicariae has been a source of controversy as some authors have claimed that the section should be extended to include species from related sections such as the Pseudocypereae, Hirtae and Paludosae. This proposal is supported by data from natural hybrids. Anatomical, biosystematic and cytological surveys in the present study suggest that there is little justification for extending the limits of Sect. Vesicariae. A consideration of published data on chromosome numbers supports this conclusion. The geographical distributions of the sections involved are also distinctive. A preliminary chemotaxonomic investigation using seed proteins has also been carried out. Attempts to produce antisera from Carex seed proteins were not very successful, but results from acrylamide gel electrophoresis were much more promising, showing a wide variety of banding patterns in different sections of Carex. Being of a preliminary nature, this survey was not, however, sufficiently complete to yield much information on sectional limits in Sect. Vesicariae.

Morphological and anatomical studies were made on C. rostrata to investigate the variation patterns occurring in this species. It would appear that a considerable part of the small-scale local variation is genetical in origin. The evidence obtained suggests that C. rostrata forms extensive clones by vegetative spread. These may be morphologically very diverse and can sometimes be distinguished visually in the field. Studies on a population sample from Grantown-on-Spey, (Moray), indicate that hybridisation between C. rostrata and C. vesicaria can occur under natural conditions and that some degree of backcrossing and introgression may be possible. Introgression is not however, considered to be a significant source of variation in either species under normal conditions.

Studies of N. American material using anatomical and morphological techniques suggest that the taxon known as C. rostrata var. utriculata should probably be raised to subspecific or possible specific rank. It is anatomically distinct from the type variety and a Principal Components Analysis (PCA) indicated that the two forms could be separated almost completely on morphological characters. The reported chromosome numbers for the two varieties also support the change of rank. No formal nomenclatural proposal has been made as further studies are required.

Use other side if necessary.

Material of C. grahamii from Britain and Scandinavia has been examined morphologically using PCA and a cytological and biosystematic study carried out on a clone from Meall nan Tarmachan in Perthshire. The chromosome number obtained was 2n = 82 or 84. This appears to be the first count for this taxon. Biosystematic and morphological evidence suggests that C. grahamii is a hybrid be- C. saxatilis and C. vesicaria as suggested by a number of authors, but it is necessary to postulate some chromosomal rearrangements to account for the observed karyotype. C. grahamii is rather heterogeneous and it is suggested that the taxon consists of a series of F_1 hybrids and backcrosses with C. saxatilis. The regular chromosome pairing seen in C. grahamii and in a C. grahamii x vesicaria hybrid would seem to indicate that C. saxatilis and C. vesicaria are very closely related. This is apparently confirmed by the similarity in the seed protein complements of the two species.